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EXPERIMENTAL STUDIES OF THE ETIOLOGY OF COMMON WARTS IN CATTLE [1]

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OCCURRENCE

The common wart, *Verruca vulgaris*, has been described as a specific type of epithelial overgrowth, nonmalignant in character. It is well known that warts are of common occurrence in animals and in man. Adults may be affected, but the young are evidently far more susceptible to these cutaneous growths.

Warts may occur on any part of the body, but in different species they seem to have certain preferred locations. In bovines the primary location of the warts depends somewhat on the age of the animal. In cows the warty growths are usually found on the teats or some other part of the udder, while in calves or yearlings the growths most frequently have their origin on parts of the head, sides of the neck, or shoulders. Occasionally the warts in young cattle may spread from the point of origin to other parts, as the sides, back, or abdomen, eventually involving large areas of the skin surface.

In calves these warty growths may become large and pendulous, sapping the strength and greatly inhibiting the growth of the animal affected. In some cases warts disappear spontaneously without apparent cause or without recourse to treatment of any kind.

ECONOMIC IMPORTANCE

As no figures are available it would be difficult even to approximate the total number of cattle affected annually with warts. It may be stated, however, that available data on the number of warty hides encountered in some of the larger slaughtering establishments indicate that the affection is probably increasing. One of the large slaughtering establishments has estimated that from 15 to 25 per cent of the hides have been found affected at certain periods, particularly during the summer season.

As to the damage caused by warts, tanners state that the final effects of such growths on the hides of cattle are somewhat similar to those observed in "grubby" hides; i. e., holes are found in the hides after tanning, rendering the parts affected practically worthless. The total number of warty hides found annually is unquestionably large, and, since these hides are greatly reduced in value, the yearly loss is sufficiently large to make it of considerable economic importance to the leather industry of the country.

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SUGGESTED CAUSES

As in other types of neoplastic growths, different theories have been advanced relative to the cause of warts. Various forms of external irritation, such as injuries, chemical irritants, etc., have been suggested as causes of warty growths. Following the theory of injury as a possible cause, some have claimed that warts are seen more frequently in branded than in unbranded cattle. Some pathologists, as Ewing (*2*),[2] for example, consider warts to be of the nature of inflammatory proliferations.

PREVIOUS EXPERIMENTS

It is commonly believed at present that warts are infectious, and the fact that investigators have been able to transmit them experimentally from animal to animal and from man to man indicates that the belief is probably well founded.

A study of the literature shows that very little experimental work has been done in this country on the transmission of warts in animals. In Europe, however, a number of experiments have been recorded. M'Fadyean and Hobday (*4*), were successful in reproducing warts in the region of the mouths of dogs by means of experimental inoculations. Royère (*5*) successfully transmitted warts in different species of animal, including the horse, dog, and calf. Schultz (*6*) mentions an experiment in which intracutaneous inoculations were made on the hands of three persons with wart material from the udder of a cow, resulting after a long period of incubation in small, warty growths at the points of inoculation.

A number of experiments have been recorded in which warts have been produced, particularly in human beings, by intracutaneous injections with filtrates of wart material. Ciuffo (*1*) produced warts in a single case in man by inoculation with wart filtrate that had passed through a Berkefeld N candle. Wile and Kingery (*7*) transmitted warts in a number of cases, in human beings, by intradermic inoculations with sterile filtrates of wart material. Kingery (*3*), continuing these experiments, was also able to produce warts in the second generation with filtrates. While experimental warts may have been produced in animals also, by inoculations with wart filtrates, the writer has been unable thus far to find any record of such experiments.

PRESENT EXPERIMENTS

Since it had apparently been satisfactorily proved by previous experiments that warts could be transmitted from animal to animal in different species, including the bovine, the experiments here described were undertaken to ascertain with what degree of frequency, or regularity, it would be possible to transmit such growths in cattle, and, more particularly, to obtain information with regard to the nature of the etiological factor involved.

A series of intracutaneous inoculations of cattle with emulsified wart material from different species was undertaken and a like number of inoculations were made with filtrates obtained from the same series of wart specimens. A limited amount of bacteriological work was also included in the experiments.

[2] Reference is made by number (italic) to "Literature cited," p. 737.

SOURCE OF WART MATERIAL

The wart specimens of bovine origin, used for inoculation purposes, were obtained from cattle-slaughtering establishments at Chicago, Ill., and Fort Worth, Tex. The specimens were received at different times and usually only one or two specimens at once. Although the points of origin of the cattle from which the specimens were obtained are not known, it is probable that these animals came from widely separated sections of the country.

EXPERIMENTAL ANIMALS

On account of their susceptibility it was thought best to use only young cattle, so far as possible, for inoculation purposes. Consequently, with the exception of two adult cows, all the animals used were calves, ranging in age from 5 to 12 months. Most of the calves, however, were from 6 to 8 months of age at the time of inoculation.

The inoculations were made from time to time as fresh wart material became available, and extended over a period of approximately one year. In all, 22 cattle were used in the experiments.

PREPARATION OF WART MATERIAL FOR INOCULATION PURPOSES

Each specimen of warty skin was first thoroughly washed under running tap water for several minutes to remove accumulations of dirt, necrotic material, etc. A number of the warts, large and small, were next clipped from the skin, including a small portion of the latter at the base of the growths. The warts were cut into small bits and thoroughly ground up, with a small amount of sterile, physiologic salt solution, in a sterile mortar. More of the salt solution was then added and the material permitted to stand for a number of hours, or overnight, at refrigerator temperature.

The fluid portion of the emulsified wart material was then poured off. A portion of this constituted the unfiltered material used for inoculation, and the filtrate was obtained in each case by passing the remaining portion of the fluid, or wart extract described above, through a bacteria-retaining filter, Berkefeld N candle, under negative pressure.

Cultural tests of the filtrates were made for sterility, aerobically and anaerobically, with uniform negative results.

TECHNIC EMPLOYED IN ANIMAL INOCULATIONS

Small areas of the skin, at the base of the ear and on the side of the neck near the shoulder were chosen for inoculation. (Fig. 1.) The hair over the area to be inoculated was removed by clipping and shaving, and the skin was washed with soap and water and rinsed with distilled water. Just before the inoculations were made the excess moisture was removed from the shaved surfaces with sterile absorbent cotton.

The inoculation procedure was the same with both the unfiltered materials and filtrates. The skin was scarified over an area from 1 to 2 inches in diameter and small amounts of the wart fluid applied to the surface. At the same time a few drops of the fluid were injected intradermically, at a number of different points, with a fine hypodermic needle.

All the experimental animals were kept isolated during these experiments.

FIGURE 1.—Experimental warts produced on calf 1399 by inoculation: A, Wart at base of ear; B, warts at both sites of inoculation

CULTURAL TECHNIC

The technic followed in culturing the warts was as follows:

The under surface of the skin at the base of the warts was rendered sterile by searing with a hot spatula. Small blocks of the wart tissue were obtained by cutting into the base of the warts with a sterile knife. The pieces of wart tissue were then transferred to both aerobic and anaerobic culture media and incubated at 37.5° C. for several days.

DISCUSSION OF EXPERIMENTAL RESULTS

For the sake of brevity the experimental results have been condensed by arranging the findings for the individual animals from month to month in tabular form. (Tables 1 and 2.) While the tables show only the monthly progress of the experiments, observations of the experimental animals were made at more frequent intervals. The tables show 11 experimental animals in each group.

By referring to Tables 1 and 2 the reader will observe that positive results were obtained in the animals inoculated with both the filtrates and unfiltered wart emulsions from five of the nine original wart specimens used for inoculation purposes. In two cases positive results were obtained only in the calves inoculated with the unfiltered material, and inoculations from specimens CH 2 and FW 3 gave entirely negative results. The two mature cows used in the experiments were inoculated from the FW 3 specimen.

Inoculations made from experimental warts on calf 1403 (fig. 2) gave positive results with both the filtrate and the unfiltered wart material, but when inoculations were made from experimental-filtrate warts on calf 1402, the second transfer of the warts was accomplished only with the filtrate. (Figs. 3 and 4.)

In comparing the inoculation results in the two groups of experimental animals the reader will note that a slightly higher percentage of positive results was obtained with the unfiltered wart material than with the filtrates. Attention may also be called to the fact that in a few cases the experimental warts appeared earlier, and seemed to show more rapid development, in the calves inoculated with the unfiltered material, than in those inoculated with filtrates from the same source. This may be taken to indicate that the filtrates were rendered less active as a result of the filtering process.

However, until we have more definite knowledge regarding the etiological factor involved, it is difficult to explain any apparent variations in the results in the two groups of experimental animals.

The experimental warts which attained a fair stage of development were typical in their clinical appearance, and those examined histologically showed a structure (figs. 5 and 6) characteristic of papillomatous growths.

In regard to the detailed studies made, it is desired to direct especial attention to only two of the experimental calves, Nos. 1402 and 1403. These calves were both inoculated from wart specimen FW 2, 1402 with the wart filtrate, and 1403 with the unfiltered wart material; this was the only instance in which both calves inoculated from the same wart specimen showed well-developed experimental warts, thus giving opportunity for comparative study of the filtrate warts and those produced with the unfiltered wart material. The culturing of the experimental warts in these two calves gave negative bacteriological results in both cases.

TABLE 1.—*Results of inoculations of cattle on ear and neck with unfiltered wart material*

Specimen	Animal No.[a]	Condition at end of— First month	Second month	Third month	Fourth month	Fifth month	Sixth month	Seventh month
CH 1	1333	Skin thickened at point of inoculation on neck.	Some evidence of growth on neck.	Flat growth about 1 inch in diameter on neck.	Growth increased; about 2 inches in diameter.	Growth decreasing in size.	Growth rapidly disappearing.	Little remaining evidence of growth.
CH 2	1336	No growth	No growth	No growth	No growth	No growth	No growth	Considered negative.
FW 1	1401	Skin thickened at point of inoculation on ear.	Ear growth larger than a pea.	Ear growth slightly increased in size.	Ear growth ½ inch in diameter; no growth on neck.	Ear growth about 1 inch in diameter.	Little change in size of growth; treated with tincture iodine.	Growth practically disappeared.
FW 2	1403	No growth	Small growth size of pea at seat of inoculation on neck.	Shoulder growth spreading; small growth on ear.	Marked increase in size of growths on ear and neck.	Growth on neck 2½ by 4 inches; ear growths 1½ by 2 inches; calf died.		
CH 3	1418	do	Skin rough at seats of inoculation.	Several small growths on ear; flat growths on neck 1 inch in diameter.	Three growths at base of ear ½ inch in size; growths on neck 2 inches in diameter.	Growths somewhat larger; treated with tincture iodine.	Growths disappeared after treatment.	
CH 4	1414	do	A few small growths on ear and neck.	Ear growths about ¾ inch in diameter; slight increase in growth on neck.	Thick growth on ear about 1 inch in diameter; four small growths on neck ½ inch in diameter.	Growths treated with acetic acid.	Growths practically disappeared.	
CH 5	1416	Skin thickened at points of inoculation.	Slight evidence of growths on ear and neck.	Several growths on ear and neck little larger than a pea.	Little increase in size of growths on ear and neck.	Growths receding in size.	Growths entirely disappeared.	
FW 3	1303 (cow)	No growth	No growth	No growth	No growth	No growth	Considered negative.	
From experimental calf 1403.	1397	do	A few minute growths at seats of inoculation.	One wart about ½ inch in diameter on ear; several pea-sized growths on neck.	Growths slightly increased in size on ear and neck.	Growths on both ear and shoulder rapidly disappearing.	Growths entirely disappeared.	
FW 4	1399	Skin thickened at points of inoculation.	Some evidence of growths on ear and neck.	A number of well-formed warts on neck and ear.	Flat, spreading growths on neck; one large and several small warts on ear.	Growth on neck about 1 inch; ear growth increasing in size.	Large, well-developed wart on ear 2 by 3 inches in size; growth on neck increased in size.	Warty growths remained about same at time animal was released.
From experimental calf 1402.	1429	No growth	No growth	No growth	No growth	No growth	No growth	Considered negative.

[a] The animals were calves unless otherwise noted.

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TABLE 2.—*Results of inoculations of cattle on ear and neck with wart filtrate*

Specimen	Animal No.[a]	Condition at end of— First month	Second month	Third month	Fourth month	Fifth month	Sixth month	Seventh month
CH 1	1334	No growth	No growth	No growth	No growth	No growth	No growth	Considered negative.
CH 2	1335	do	do	do	do	do	do	Do.
FW 1	1400	do	Skin thick and rough on neck.	Small, flat growth about 1½ inches in diameter.	Flat, spreading growth on neck 2 inches in diameter.	Growth no larger; no growth on ear.	Growth rapidly receding in size.	Growth has practically disappeared.
FW 2	1402	do	Indications of growth at seat of inoculation on neck.	Flat growth on neck 1 inch in diameter; a few small growths on ear.	Growth on neck increasing in size and thickness.	Well-developed, warty growth on neck 2 by 4 inches; ear growths remained small.	Little change in size of growths; calf slaughtered.	
CH 3	1417	do	No growth	Thickened and rough appearance of skin at seat of inoculation.	A number of small warty growths on neck and at base of ear.	Growths no larger than a pea; appear to be receding in size.	Only two small growths remaining on neck; one on ear.	Growths entirely disappeared.
CH 4	1413	do	A few small growths at seat of inoculation on ear.	Growths on ear disappearing; no growth on neck.	Growths entirely disappeared.			
CH 5	1415	do	No growth	Skin appeared slightly nodular at points of inoculation.	A number of pea-sized growths at base of ear and on neck.	No apparent change in size of growths.	Growths on ear and neck rapidly disappearing.	Do.
FW 3	1269 (cow)	do	do	No growth	No growth	No growth	Considered negative.	
From experimental calf 1403.	1398	do	Some evidence of very small growths on ear and neck.	A number of growths no larger than a pea at both seats of inoculations.	No increase in size of warty growths.	All growths practically disappeared.	No remaining evidence of growths.	
FW 4	1427	do	No growth	No growth	No growth	No growth	No growth	Considered negative.
From experimental calf 1402.	1428	Skin appeared thick over inoculated areas.	Some evidence of growths on ear and neck.	Numerous small growths on neck; a few at base of ear.	Growths on neck larger, covering area of 2 by 3 inches; ear growths remained small.	Calf found dead; slight increase in size of growths on neck.		

[a] The animals were calves unless otherwise noted.

FIGURE 2.—Experimental warts produced on calf 1403 by inoculation. A, Warts on ear; B, well-developed growth at site of inoculation on neck. (Natural size)

FIGURE 3.—Experimental warts on the neck of calf 1402 produced by inoculation with wart filtrate. (Natural size)

FIGURE 4.—Second generation of filtrate warts on neck of calf 1428, which was inoculated with filtrate of experimental warts from calf 1402. (Natural size)

FIGURE 5.—Section of wart, specimen CH 1, showing typical structure of bovine warts used in original inoculation. × 80

FIGURE 6.—Section of experimental wart from calf 1403, produced by inoculation with unfiltered wart material. × 80

After successfully producing experimental warts with sterile wart filtrates the writer saw no practical value in continuing bacteriological studies of such growths.

A histological comparison of warts from cases 1402 and 1403 shows some rather interesting differences. (Figs. 7 and 8.) In case 1402 there is a marked proliferation of the fibrous structure, the vessels are more numerous, and there is a very noticeable hyperkeratosis. The epithelial tissue is rather limited in this case.

Case 1403 shows typical hypertrophy of the papillae, the increase in the epithelial tissue being very noticeable. The cornified epithelium, it will be noted, is rather scant in this case. The histological variations noted in these two cases are rather interesting but not easily explained.

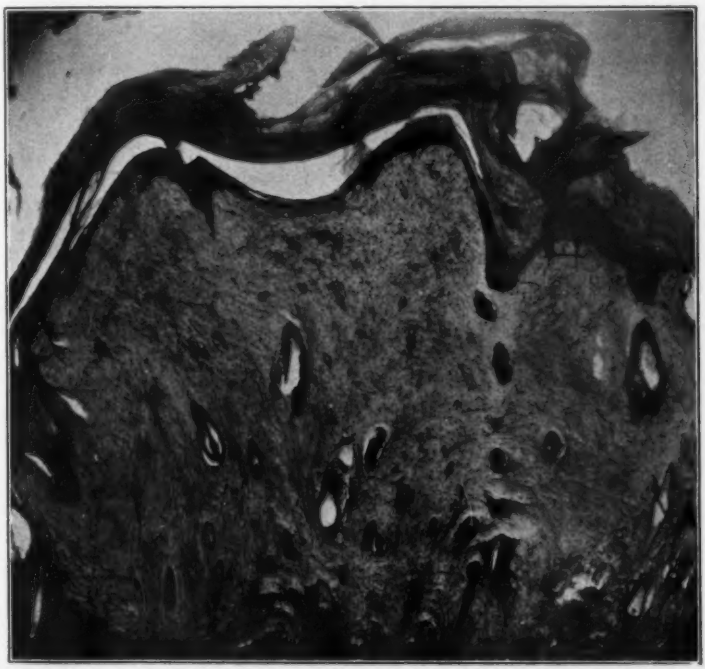

FIGURE 7.—Section of experimental wart from calf 1402, produced by inoculation with wart filtrate. × 40

While it is generally conceded that warts may be transmitted through experimental inoculations, there are some who question the infectious nature of these growths. Ewing (*2*) states: "There are numerous clinical observations suggesting the contagiousness of warts, but on analysis they can be dismissed as examples of the transfer of peculiar, chemical irritants, or exposure to the same irritant, or the actual transfer of proliferating epithelial cells." The possibility of producing warty growths by transplanting proliferating epithelial cells is, of course, entirely eliminated in those cases of experimental warts produced by inoculations with filtrates as shown in Table 2.

That any possible chemical substance causing an original wart should remain sufficiently toxic or irritating, after being greatly diluted, subjected to the filtering process, etc., prior to each inoculation, to cause definite warty growths, seems to the writer to be somewhat questionable, especially so when the second generation of such growths is produced.

On the other hand, Wile and Kingery (7) are of the opinion that "the clinical evidence in favor of an infectious agent in the causation of warts is extremely suggestive," and add that the appearance of

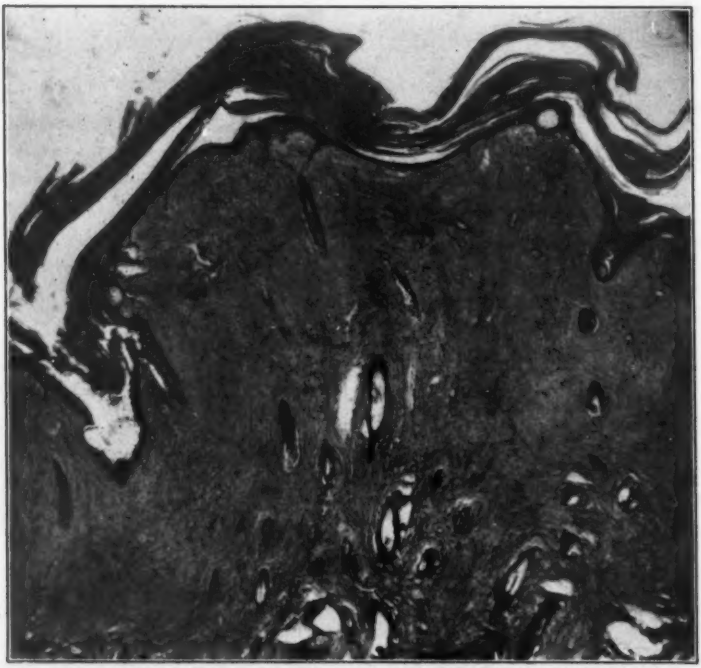

FIGURE 8.—Another section of experimental wart from calf 1402 showing a histological structure slightly different from that shown in Figure 7. × 40

the so-called daughter warts following larger warts, the appearance of warts on contiguous surfaces, and the occurrence of groups of individual warts lend color to this view of the contagious nature of common warts. The results of the present experiments seem to be more in accord with the view of the last-mentioned investigators, and tend to substantiate the belief that the causative agent in common warts of cattle partakes of the nature of a filterable virus.

SUMMARY AND CONCLUSIONS

Definite positive results were obtained in 15 out of a total of 22 cattle inoculated with filtered or unfiltered wart material. Eight of the calves giving positive results were inoculated with the unfiltered

wart material and seven with the wart filtrates. The results obtained indicate that common warts in cattle can be experimentally transmitted to bovines under 1 year of age with a fair degree of regularity.

It was also demonstrated that filtrates of bovine warts, proved by cultural tests to be free from cultivable microorganisms, were capable of producing papillomata when inoculated intracutaneously into healthy cattle, and that warts produced by filtrate inoculations may be successfully transmitted in the second generation.

The results of the filtrate experiments seem to justify the conclusion that the causative factor in common warts of cattle is probably of the nature of a filterable virus.

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CHANGES IN THE PECTIC CONSTITUENTS OF APPLES IN RELATION TO SOFTENING[1]

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INTRODUCTION

One of the principal changes that takes place in apples as they ripen on the tree and in storage is the softening of the flesh of the fruit. In recent years many studies have been made on the ripening and storage of apples (*10*),[3] and in these the softening of the fruit has been given major consideration. In general, it has been found that there is a gradual, though somewhat irregular, softening of the fruit on the tree as it becomes mature. This softening continues in storage and is the outstanding change that takes place during the process of ripening, the fruit changing from the hard unripe condition to the relatively soft, eating-ripe condition, and finally to the very soft, mealy, overripe condition. The rate at which softening takes place in storage depends primarily upon the temperature at which the apples are kept. For a number of varieties the rate of softening at 40° F. was found to be slightly more than double that at 32°, whereas that at 50° was slightly less than double that at 40°, and that at 60° was nearly double the rate at 50°.

In these studies (*10*) a fruit pressure tester (*9*) was used to measure the firmness of the apples. This tester records the pressure in pounds necessary to force a plunger seven-sixteenths of an inch in diameter into the fruit a distance of five-sixteenths of an inch. In penetrating the fruit the tester crushes some of the cells and separates and pushes aside others. The firmness of the apples as measured by the pressure tester is probably due principally to three factors—the size of the cells and intercellular spaces, the thickness of the cell walls, and the ease with which the cells separate. The last two factors are of particular interest in relation to the studies herein reported, as the thickness of the cell walls depends on the thickness of the cellulose or substance of which they are composed and upon the thickness of the layer of cementing substance or pectic material of the middle lamella, while the ease with which the cells separate depends upon the amount of pectic material cementing them together. All three factors might play a part in the softening of the fruit on the tree. In storage, however, there would be no further increase in the size of the cells and intercellular spaces, and softening should be due to changes in the thickness of the cell wall and the ease with which the cells separate.

The studies reported in this paper were undertaken to obtain a better understanding of the underlying chemical changes that take place in apples during the softening process.

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[3] Reference is made by number (italic) to "Literature cited," p. 746.

REVIEW OF LITERATURE

In the recent literature dealing with the pectic material in fruit, particularly in apples, the following pectic substances have been recognized: Pectin, which is water soluble and is derived from protopectin probably by enzyme action; protopectin or pectose, which is water insoluble; and the pectic substance of the middle lamella, which is also water insoluble. The total pectic substances really include all three of these, but in this paper only the pectin and protopectin are included as the pectic substance of the middle lamella was not determined.

Several investigators have found softening in fruit to be associated with changes in the pectic material in the cell walls and middle lamella. Carré (*3, 4*) made extensive studies of the pectic materials of apples in storage. She associated ripening in storage with an increase in pectin and a corresponding decrease in protopectin (pectose), the total pectic materials remaining constant. In the latter stages of storage in which the fruit becomes mealy and breaks down she observed a breaking down of what she termed the "pectic substance of the middle lamella." She found that during this period there is a temporary increase in protopectin, which again decreases. These changes were observed to take place more rapidly at higher temperatures. Similar results were observed by Carré and Horne (*6*) when microchemical methods were used. With peaches, Appleman and Conrad (*1*) found that there was a decrease in protopectin and a corresponding increase in pectin with ripening in storage, the rate of these changes increasing with the temperature. When the fruit ripened on the tree very little change was noted in the pectic materials. Emmett (*8*), working with pears, obtained similar results. At 1° C. the development of pectin was greatly retarded, as was also the softening of the fruit. Appleman and Conrad (*2*) found an increase in pectin and a decrease in protopectin with increased maturity of tomatoes while on the vine. In canned tomatoes a close relationship existed between the amount of disintegration and the ratio of pectin to protopectin.

From the investigations discussed above it would seem that the cell walls are thickened and cemented together by an insoluble pectic material, usually protopectin. As softening takes place the protopectin is changed to a soluble form, thus permitting the cells to separate readily and decreasing the thickness of their walls. These changes are accelerated by an increase in temperature.

In the investigations previously cited no measurements were made of the firmness of the fruit, except with tomatoes (*2*), under the various conditions of the experiments. It was the purpose of the present investigation to determine to what extent the changes in the pectic materials of apples are responsible for the softening of the fruit as measured by the pressure tester.

METHODS

The determination of the pectic materials (pectin, total pectic material, and protopectic) was carried out according to the method described by Carré and Haynes (*4, 5*) with some modifications.

A composite sample of at least 20 apples was used for each determination. The firmness of the fruit was first determined by means

of the pressure tester. The fruit was then pared and cored, and the flesh was run through a sampling press (7) which macerated the tissue to a fine pulp.

The soluble pectin was determined in two ways, as follows:

1. Samples of 100 gm. of pulp were frozen overnight. After thawing, the juice was pressed out through muslin. The pulp was then repeatedly washed with cold water until no appreciable amount of pectin remained. Preliminary investigations had shown that seven washings with 150 to 200 milliliters of water were sufficient to accomplish this. The juice and the first washing were combined, as were also each additional two washings. They were then brought to a boil, cooled, and made to a 500-ml. volume. After filtering, aliquots of sufficient size to yield if possible 0.02 to 0.03 gm. of calcium pectate were transferred to beakers, diluted to 300 ml., made alkaline with 100 ml. of 0.1 normal sodium hydroxide, and allowed to stand overnight. They were then acidified with 50 ml. of normal acetic acid, and the calcium pectate was precipitated by the addition of 50 ml. of molar calcium chloride. The precipitate was then brought to a boil, filtered, and washed with hot water until free from chlorides. The precipitate was washed back into the beaker, boiled, filtered again, and washed. It was then washed into a small tared beaker, dried, and weighed.

2. Portions of the pulp were frozen overnight. After thawing, the juice was pressed out through muslin. Definite volumes, 100 ml. of the juice were brought to a boil, cooled, and made to a 500-ml. volume. After filtering, aliquots of sufficient size to yield 0.02 to 0.03 gm. of calcium pectate were used, and from this point the determinations were carried on as in the first method.

In Tables 1 and 2 the two methods are compared. The soluble pectin in the pulp was obtained by the first method, while that in the juice was obtained by the second. As the results by both methods were practically the same, the second method, which was much shorter, was used in the later determinations.

The determination of the total pectic substances was carried on as follows: The juice from 100-gm. samples of pulp was pressed out through muslin, and the pulp was washed with cold water to remove the organic acids and most of the soluble pectin. The juice and the wash water were combined, brought to a boil, cooled, and made to volume. The residue was then transferred to a flask, covered with 1/75 normal hydrochloric acid, and boiled for an hour under a reflux condenser. The solution was then pressed out through muslin and made to a 500-ml. volume. Extraction of the residue with 1/75 normal hydrochloric acid was repeated twice. After filtering, aliquots of each extraction of sufficient size to yield, if possible, 0.02 to 0.03 gm. of calcium pectate were used, and the determinations were carried on from this point as for soluble pectin.

Protopectin was obtained by subtracting the soluble pectin from the total pectic substance.

EXPERIMENTAL DATA

SOFTENING ON THE TREE IN RELATION TO PECTIC CHANGES

The data on the relation of softening of fruit while on the tree to the changes in the pectic constituents are presented in Table 1 and Figure 1. The amount of soluble pectin in apples as they ripen

on the tree is very small and remains practically constant. As the fruit softens there is a gradual decrease in the percentage of protopectin and a corresponding decrease in total pectin in the Ben Davis apples. In the Jonathan, however, the pectic changes during the first period are negligible even though there is a softening of 6.5 pounds. During the second period the changes correspond to those in the Ben Davis apples. Although the percentage of protopectin decreases, it is probable that the actual amount does not decrease but seems to be less because of the increased size of the fruit. Softening on the tree appears to be associated in part with the apparent decrease in the protopectin content. Other factors, such as increase in the size of the cells and intercellular spaces, may be the principal ones responsible for softening in the rapidly growing fruit.

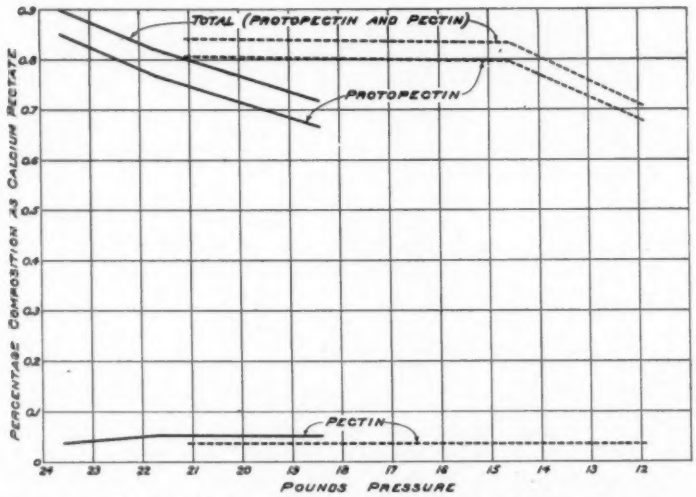

FIGURE 1.—Pectic changes during softening of apples on the tree. Solid line represents Ben Davis in 1926; broken line, Jonathan in 1927

TABLE 1.—*Softening of apples on the tree in relation to changes in pectic constituents*

[Pectic substances expressed as calcium pectate in percentage of fresh weight]

Variety of apple and date of test	Pressure test	Soluble pectin		Protopectin	Total pectic substances
		In juice	In pulp		
Ben Davis:	*Pounds*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sept. 1, 1926	23.6	0.038	0.048	0.851	0.899
Sept. 17, 1926	21.7	.052	.053	.765	.818
Oct. 14, 1926	18.4	.051	.047	.608	.715
Jonathan:					
July 22, 1927	21.1	.033		.807	.840
Aug. 22, 1927	14.6	.037		.796	.833
Sept. 14, 1927	11.9	.034		.673	.707

SOFTENING IN STORAGE AT 32° F. IN RELATION TO PECTIC CHANGES

Ben Davis, Winesap, and two pickings of Jonathan were used to determine the relation of softening in storage at 32° F. to pectic changes. As softening took place there was a gradual increase in total pectic materials (Table 2 and fig. 2) in Ben Davis and Winesap apples, whereas in Jonathan the total pectic materials remained fairly constant but showed a slight decrease. There was a gradual decrease in protopectin with softening in all varieties except for the last determination on Ben Davis and Winesap apples, when there was an increase. These increases may have been due in part to loss of water by the fruit, which showed considerable shriveling. It is more probable, however, that they were due to the formation of protopectin from the so-called pectic substance of the middle lamella

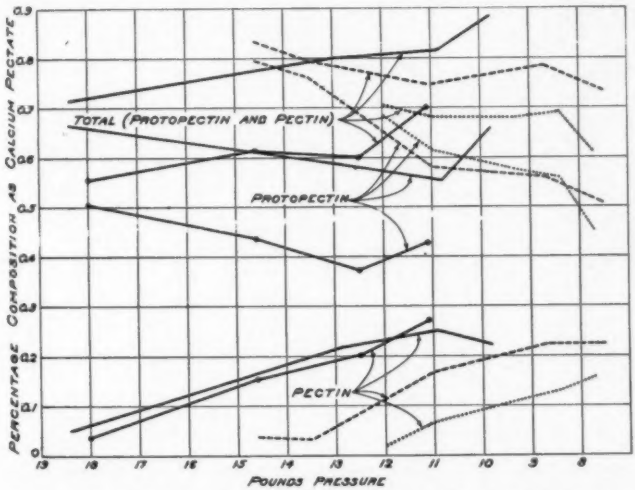

FIGURE 2.—Pectic changes and softening of apples in storage at 32° F. Broken line represents Jonathan I in 1927; dotted line, Jonathan II in 1927; solid line, Ben Davis in 1926; dot-solid line, Winesap in 1926

described by Carré, as the increases in both protopectin and total pectic substances were greater than would be expected, from loss of water, especially as there was no corresponding increase in pectin.

Fruit stored at 32° F. showed a regular increase in soluble pectin, due principally to the hydrolysis of protopectin. In contrast, fruit allowed to ripen on the tree showed no change in soluble pectin during the softening process.

There was no relation between the firmness of the three varieties and their pectic constituents. Jonathan, which was much softer when picked than Winesap and Ben Davis, had a higher protopectin and total pectin content.

The total pectic material and soluble pectin were at all times lower in the later picking of Jonathan than in the earlier picking, while the protopectin was nearly equal in the two pickings at the various stages of softening.

TABLE 2.—*Softening of apples in storage in relation to changes in pectic constituents*

[Pectic substances expressed as calcium pectate in percentage of fresh weight]

Variety of apple and date of test	Temperature	Pressure test	Soluble pectin		Protopectin	Total pectic substances
			In juice	In pulp		
Ben Davis:	°F.	Pounds	Per cent	Per cent	Per cent	Per cent
Oct. 14, 1926 *	32	18.4	0.051	0.047	0.668	0.715
Jan. 31, 1927		12.9	.216	.214	.587	.801
Apr. 6, 1927		10.9	.250	.262	.532	.814
July 18, 1927		9.8	.221	.228	.656	.884
Winesaps:						
Oct. 27, 1926 *	32	18.0	.036	.060	.504	.564
Feb. 14, 1927		14.6	.154	.175	.436	.611
Apr. 22, 1927		12.5	.202	.231	.371	.602
July 22, 1927		11.1	.263	.275	.425	.700
Jonathan I:						
Aug. 22, 1927 *	32	14.6	.037		.796	.833
Oct. 3, 1927		13.5	.032		.760	.792
Nov. 14, 1927		11.0	.166		.579	.745
Dec. 21, 1927		8.7	.223		.561	.784
Mar. 1, 1928		7.5	.225		.508	.733
Jonathan II:						
Sept. 14, 1927 *	32	11.9	.034		.673	.707
Oct. 14, 1927		12.0	.018		.687	.705
Nov. 16, 1927		11.0	.063		.618	.681
Dec. 21, 1927		9.3	.107		.574	.681
Mar. 3, 1928		8.4	.130		.560	.690
May 2, 1928		7.7	.158		.452	.610
Jonathan I:						
Aug. 22, 1927 *	40	14.6	.037		.796	.833
Sept. 15, 1927		13.5	.055		.705	.760
Oct. 5, 1927		9.7	.159		.627	.786
Oct. 24, 1927		8.5	.229		.447	.676
Dec. 17, 1927		7.7	.252		.435	.687
Jonathan I:						
Aug. 22, 1927 *	50	14.6	.037		.796	.833
Sept. 8, 1927		13.2	.048		.737	.785
Sept. 20, 1927		8.7	.237		.454	.691
Oct. 26, 1927		7.2	.278		.384	.662
Jonathan I:						
Aug. 22, 1927 *	60	14.6	.037		.796	.833
Aug. 26, 1927		14.8	.015		.730	.745
Aug. 31, 1927		14.2	.017		.681	.698
Sept. 9, 1927		9.3	.185		.549	.734
Sept. 30, 1927		7.5	.270		.408	.678

* Date picked and placed in storage.

SOFTENING IN STORAGE AT VARIOUS TEMPERATURES IN RELATION TO PECTIC CHANGES

Fruit from the second picking of Jonathan was placed in storage at 32°, 40°, 50°, and 60° F. Determinations were made at intervals, and the data are presented in Table 2 and Figure 3.

There was some irregularity in the percentage of total pectic substances and protopectin at the various temperatures. In general, there was a tendency for the amount of total pectic substances to decrease slightly with softening. There was a decrease in protopectin with softening, as shown in Figure 3, from more than 0.8 per cent at the start with a pressure test of 14.6 pounds to slightly less than 0.45 per cent at 40°, 50°, and 60°, and 0.53 per cent at 32° F., when the fruit tested 8 pounds. A very consistent increase in soluble pectin occurred at all temperatures, with softening from 0.04 per cent at the start to about 0.25 per cent when the fruit tested 8 pounds.

That the rate of softening is proportional to the rate at which the protopectin is converted into pectin is shown in Table 3. In storage at 32° F., 100 days were required for the fruit to soften from a pressure test of 14.6 pounds, the hard-ripe condition at picking time, to 10

pounds, which represents a full-ripe condition. At the same time the protopectin decreased from 0.796 to 0.585 per cent and the pectin increased from 0.037 to 0.195 per cent. Using the conditions found at 32° storage as standards for comparison with those at the other temperatures, it was found that at 40° it required 42 days to reach the same condition of softness, and 44 and 51 days, respectively, for the corresponding changes in the protopectin and pectin to take place. At 50° it required 29 days for the pressure test to become 10 pounds, and 27 and 31 days, respectively, to reach 0.585 per cent protopectin and 0.195 per cent pectin, while at 60° the corresponding changes took place in 16, 15, and 19 days, respectively.

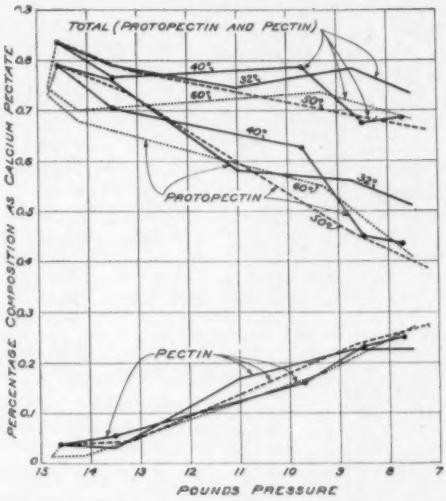

FIGURE 3.—Pectic changes and softening of Jonathan apples in storage at different temperatures. Solid line represents 32° F.; circle-solid line, 40°; broken line, 50°; dotted line, 60°

TABLE 3.—*Comparison of rate of softening of apples in storage with rate of change in percentage of protopectin and pectin at different temperatures*

Temperature		Days required to reach—		
°F.	°C.	Pressure test of 10 pounds	Protopectin content of 0.585 per cent	Pectin content of 0.195 per cent
32	0	100	100	100
40	4.4	42	44	51
50	10.0	29	27	31
60	15.5	16	15	19

At the time the first determination was made on the fruit stored at 60° F. there was a slight increase in firmness which was associated with a slight decrease in pectin and a considerable decrease in protopectin and total pectic substances. With this exception, the changes in the pectic constituents at the various temperatures were proportional to the rate of softening at each temperature.

SUMMARY AND CONCLUSIONS

The relation between the softening of the fruit on the tree and in storage, as measured by a mechanical pressure tester, and changes in the pectic constituents of the fruit was studied, and from the data obtained the following conclusions seem justified:

Softening on the tree as apples approach maturity is to some extent associated with a decrease in the percentage of protopectin and a corresponding decrease in total pectic substances, whereas the pectin, which is present in very small amounts, remain constant. Softening on the tree can not be entirely accounted for by changes in the pectic constituents.

The relative firmness of different varieties of apple likewise can not be accounted for by differences in their pectic constituents.

Softening in storage is apparently due to the conversion of insoluble pectic substances, principally protopectin, into soluble form. The rate of conversion at different temperatures is proportional to the rate of softening.

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THE TENDENCY OF THE CROWN-GALL ORGANISM TO PRODUCE ROOTS IN CONJUNCTION WITH TUMORS[1]

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INTRODUCTION

In his early work with the crown-gall organism, *Bacterium tumefaciens*, Erwin F. Smith made definite reference to the production of roots by the apple strain of that organism (*20*).[2] Later, when writing about the analogy of plant to animal cancer through the formation of teratomata (*17, 18*), he described and pictured many leafy shoots on or along the sides of tumors produced by inoculating tobacco, Bryophyllum, and geranium with several different strains of the organism. He attributed these abnormal leafy shoots, flower buds, and root formations to the kind of tissues inoculated, together with the growth conditions of the plant at the time of inoculation, and he thought that the diffusible products of the bacteria within a growing tumor could stimulate surrounding uninoculated tissues to multiply. His interest in showing the analogy of plant to animal tumors influenced him to work along the more striking line of shoot production.

The stimulation to root production by the various strains of *Bacterium tumefaciens* has been somewhat overlooked. This statement does not refer to the apple strain, however, which has been recognized since 1908 as a strain that produces a mass of roots (fig. 1) when it enters a wounded or poorly grafted apple root. Some investigators maintain that there are two types of apple strain, one that produces only tumors and another that produces only masses of roots. In the writer's opinion, which is based on years of observation and investigation, there is only one apple strain, and in this paper, which deals largely with the apple strain, the crown gall and infectious hairy root of apple are considered as the same disease, which manifests itself as a tumor, a mass of roots, or a combination of both. It happens that in those cases where roots occur in clumps without a definite tumor, inconspicuous swellings are usually present.

REVIEW OF LITERATURE

In his work with crown gall, which embraced hundreds of experiments in orchards and nurseries, Hedgcock (*3*) in 1905 established the pathological effect of crown gall on orchard trees. He produced many cross infections with minced galls from peach, apricot, almond, apple, chestnut, and other trees by inserting pieces in wounds on seedling trees planted in sterile soil, and found that by this method he could produce infection readily on some hosts. The apple, however, was infected with difficulty, and when galls of the apple were transferred to other fruit trees infection occurred only on a limited

[1] Received for publication Apr. 1, 1929; issued November, 1929.
[2] Reference is made by number (italic) to "Literature cited," p. 765.

FIGURE 1.—Hairy root on Windsor apple from Nebraska. Photographed November, 1927. One-half natural size

number of hosts (*4*). Hedgcock noted a marked difference in the susceptibility of different varieties of apple trees to crown gall and he found that infection on some varieties tended to the gall type and on others to the hairy-root type. He did not establish the cause of these outgrowths on fruit trees. He reported that not all the malformations on apple trees were due to an infectious agent, but that many were caused by poor grafting unions which resulted in an excess of callus formation (*5*).

In 1907 the bacterial origin of the crown gall on the Paris daisy was established by Smith and Townsend (*19*), and the causal organism was named *Bacterium tumefaciens*. In 1911 it was further determined that the organism had the ability to cause tumor growths on plants in many unrelated families. One of these hosts was the apple, on which the disease was manifested by a definite gall or a small outgrowth from which a mass of roots projected (*20*).

The malformations on apple stems reported years ago in England are considered by horticulturists there and by Charles F. Swingle in the United States as varietal characteristics. They have been designated as burr knots (*2, 8, 21*).

The writer made many attempts to isolate *Bacterium tumefaciens* from burr knots on apple but without success (*1*). Undoubtedly there are outgrowths on apple stems caused by *Bact. tumefaciens*, but the writer believes that the burr knot or stem tumor of apple is produced by some other agent.

Studies of outgrowths on grafted apple trees and proof of the production of callus formation without the intervention of *Bacterium tumefaciens*, and other related crown-gall investigations, have been carried on by Riker, Keitt, Melhus, and Muncie, working alone or in groups (*6, 7, 9, 10, 11, 12, 13, 14*). They induced overgrowths on grafted apple trees at the scion tips, using aseptical methods with the stock and scion cuttings to reduce as far as practicable or completely eliminate the presence of any organism. In their isolation studies with apple galls they obtained what appeared to be *Bact. tumefaciens* colonies on poured plates, but except in a very few cases inoculations with subcultures failed to produce the outgrowths necessary for proof. The percentages ran low even when isolations were made from 100 to 200 and more apple galls. The plants used for testing the pathogenicity of these isolations were mostly tomato, but tobacco and geranium were also used. These three are all hosts on which it is difficult to produce tumors with the apple strain, although tumors are produced readily on them with some other crown-gall strains. In fact, there is no quick way of testing the pathogenicity of *Bact. tumefaciens* isolated from apple crown gall. The Paris daisy, *Impatiens balsamina*, and string-bean stems have been found by the writer, as noted elsewhere in this paper, to become infected with the apple strain in less time than is required to produce outgrowths on the apple stems, but usually three to seven weeks must be allowed even for these hosts.

Recently Siegler (*16*) also isolated *Bacterium tumefaciens* from galls on apple trees and proved its pathogenicity by producing roots and outgrowths on apple and various other hosts. His isolations also showed the slowness and inadequacy of the apple strain in producing definite roots and sizable tumors on tobacco, geranium, and tomato plants.

As shown in some of the publications cited, the malformations on apple trees are classified under several names, the one called noninfectious hairy root being considered by some workers the most prevalent type.

Riker, Banfield, Wright, and Keitt (*15*) recently isolated from apple crown gall an organism which produced roots when inoculated into apple trees but which failed to produce infection on tomato plants. They inoculated 15 strains isolated from 15 different sources into young apple shoots, and all produced roots; the organism was reisolated from the enlargements at the bases of these roots. This the writer considers the typical apple strain of crown gall, which will not infect tomato, or at least will not produce a conspicuous infection, until it has passed through and been isolated from another host. What Riker and his associate call a root-stimulating organism, as though it were unrelated to crown gall, is the apple strain, which in reality is a root-stimulating organism. The apple strain showed this tendency in 1908 when the writer first isolated it from both galls and hairy roots on apple trees. It possesses this strain difference just as it also possesses similarities to other strains of crown gall, and the writer does not think it should be placed under any new classification.

THE APPLE STRAIN OF BACTERIUM TUMEFACIENS

ROOT-PRODUCING ABILITY

The general tendency of the crown-gall organism isolated from an apple tree naturally infected with hairy root is to produce roots when inoculated into an apple tree (figs. 2, A*a*, and 3, B), but it can produce definite tumors (fig. 2, A*b*) and some flat outgrowths covered or partially covered with root primordia (fig. 2, D).

This ability of the apple strain to stimulate the production of roots is not confined to infections on the apple tree, for it readily stimulated root production on stems of the Paris daisy (fig. 4, A*a*, A*b*), Bryophyllum (fig. 5, B*a*), and sugar-beet root (fig. 6, A). Not every successful inoculation produces roots, and a tumor may form without roots, as is shown on the Bryophyllum. (Fig. 5, B*b*.) Sugar beets inoculated with the apple strain produce roots as the usual type of infection, but frequently there are tumors of various sizes present. Figure 6, B, shows a definite tumor produced on a sugar beet inoculated with the hop strain and, growing in the same bed, a sugar beet inoculated at the same time with the apple strain. (Fig. 6, A.)

Tumors without roots were produced on rose and bean plants by the same culture that produced roots on daisies and sugar beets. Inconspicuous root primordia were present on the tumors, but the surfaces were not covered by them.

An organism isolated from a natural rootless tumor of apple produced roots and definite sizable tumors on Paris daisy stems. (Fig. 5, D and C.) It also produced definite tumors on apple trees. (Fig. 3, C.) An organism isolated from an induced tumor (without roots) on an apple tree can produce both tumors and roots. This was proved by inoculating sugar beets with such an isolation. Masses of roots occurred at the inoculation places on eight of them, and in addition tumors occurred on four.

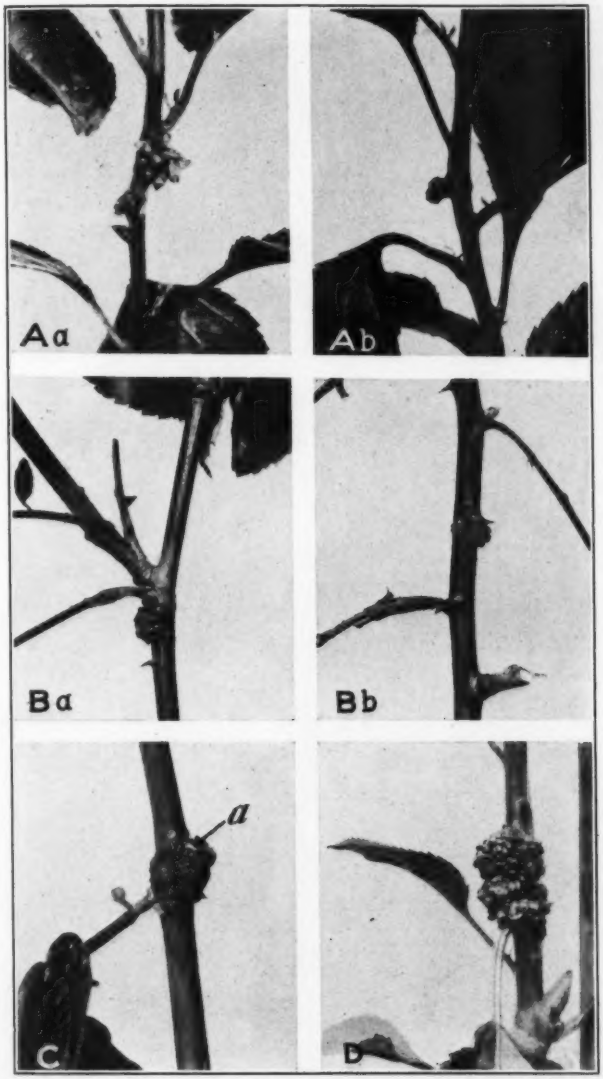

FIGURE 2.—Inoculations of various hosts with the organism isolated from an apple hairy root. All natural size: A, Seedling apple trees, two months after inoculation; Aa, tumor with roots; Ab, tumor, without roots. Ba and Bb, Rose stems, five months after inoculation. C, Stem of string bean, three months after inoculation. Small white root, *a*, developed after plant had been kept in refrigerator at 10° to 14° C. for 20 hours. D, Seedling apple tree, seven months after inoculation

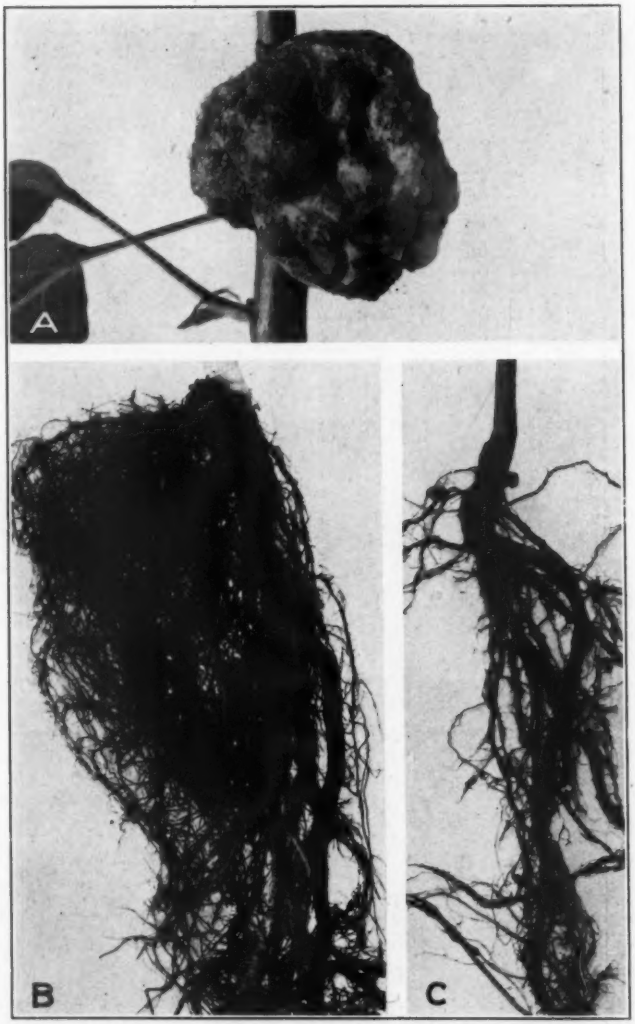

FIGURE 3.—A, The peach strain of *Bacterium tumefaciens* inoculated into a growing stem of an apple tree. Time, five months. Three-fourths natural size. B, Apple tree inoculated at crown with organism isolated from the hairy-root type of infection, showing hard tumor and masses of roots. Time, nearly five months. Reduced in size. C, Apple tree inoculated at crown with organism isolated from tumor on apple without the root masses, and in this case producing only tumors. Time, two months. Reduced in size. The same isolation produced masses of root primordia on Paris daisy stems (fig. 5, D) and a definite tumor with roots on Paris daisy at crown (fig. 5, C)

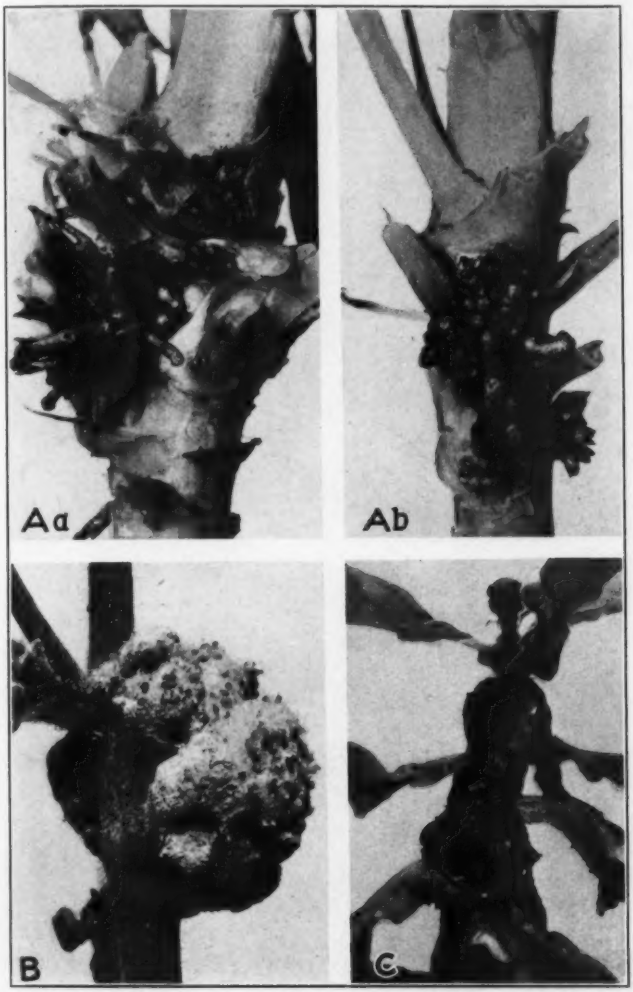

FIGURE 4.—Aa, and Ab, Paris daisy stems inoculated with the organism isolated from hairy root of Windsor apple shown in Figure 1, November 23, 1927. Photographed March 3, 1928. × nearly 2. B, Tobacco stem inoculated with rose strain of *Bacterium tumefaciens* showing tumor with root primordia. Time, two months. × nearly 2. C, Tobacco stem inoculated with peach strain of *Bact. tumefaciens* showing tumor with roots. Time two months. Natural size

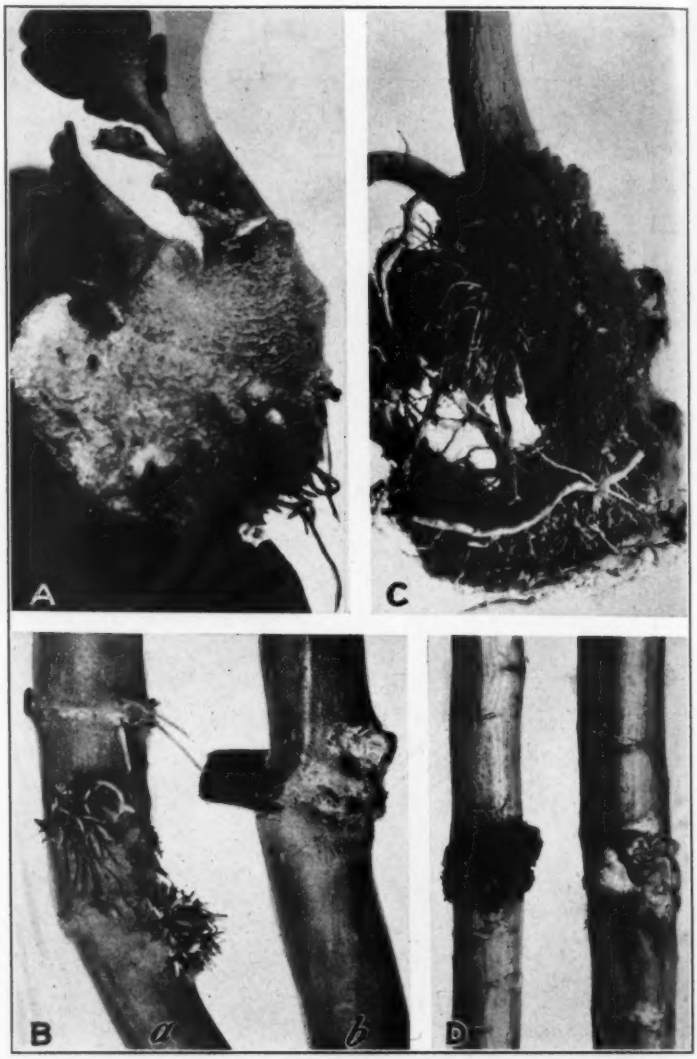

FIGURE 5.—A, *Bryophyllum pinnatum* inoculated with the hop strain of *Bacterium tumefaciens*, showing tumor and roots. Time, three months. × nearly 5. B, *Bryophyllum pinnatum* inoculated with the apple hairy-root strain of *Bact. tumefaciens*. The stem with the tumor and no definite roots, Bb, was inoculated with the original isolation colony. The stem with roots, Ba, was inoculated with a reisolation colony on the same date. Time of both, five months. Natural size. C, Tumor with roots on Paris daisy produced by inoculating the crown with an organism isolated from a definite tumor on apple (in distinction from hairy root). Time, 10 months. Natural size. Figure 3, C, shows the same isolation inoculated into apple crown. D, Tumor and masses of root primordia produced by inoculating stems of Paris daisy with the same organism used to produce C. Time, eight months. Natural size

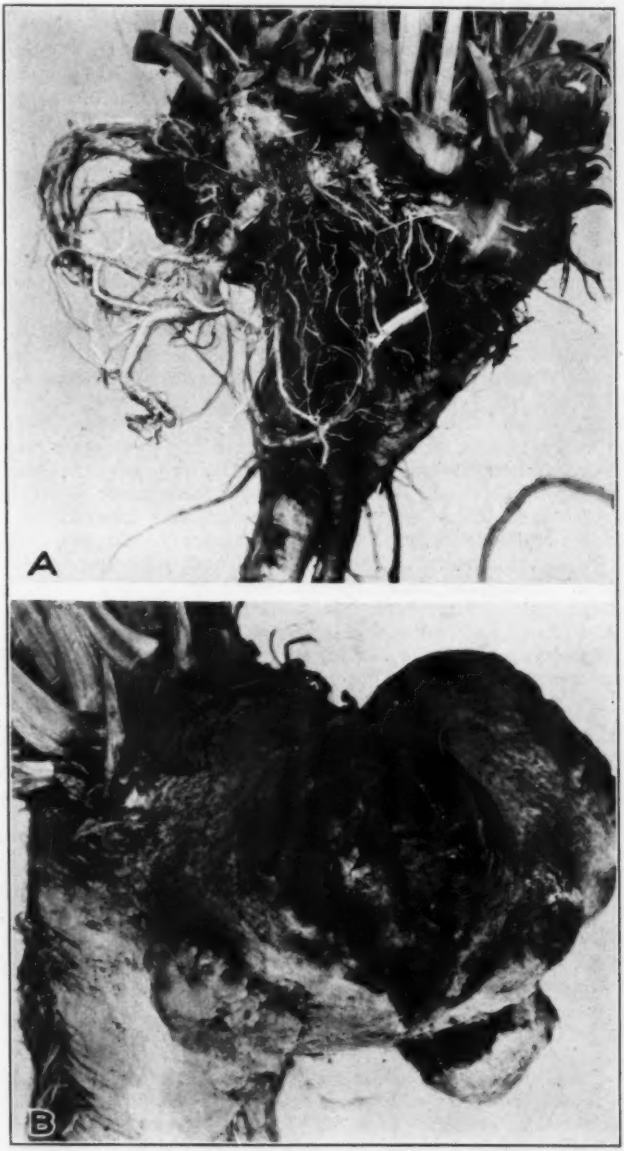

FIGURE 6.—A, Sugar beet inoculated with the apple hairy-root strain of *Bacterium tumefaciens*, March 27, 1928. Photographed June 23, 1928. Tumors and roots produced. B, Sugar beet inoculated with the hop strain at the same time and photographed on the same date as A. Only a tumor produced. Natural size

THE APPLE TREE AS A HOST

The apple tree is a less favorable host to the crown-gall organism than the peach and apricot, on whose tender cortex tumors form more readily. But it is not entirely due to the less tender tissues of apple that tumors or hairy roots form more slowly on it than on peach and apricot. The appearance of tumor growths on rapidly growing apple seedlings inoculated with the apple strain usually requires two months, which is twice as long, or even longer, than the time required for tumors to appear on peach or apricot trees inoculated with the peach and apricot strains of *Bacterium tumefaciens*. Even the active peach strain when inoculated into apple trees is a slow tumor producer, but it is much more rapid than the apple strain. (Fig. 3, A.) The slowness of the peach strain in producing tumors on apple trees may be due to the more acid condition of the apple stem, as indicated by the pH, 4.6. The peach stem has a pH value of 4.8. The tests of both peach and apple stems were made near the close of the summer, however, and there might have been a wider difference in the pH values had the tests been made at some other time of the year.

The slowness of the apple strain in producing tumors on its native host is characteristic of its action on the other hosts to which it is infectious. When young, quickly growing daisy plants are inoculated with the apple strain by using subcultures soon after isolating, the tumors or roots do not appear even as swellings until six or seven weeks have elapsed, and growth continues slowly. Figure 4, A*a* and A*b* show daisy stems three and one-half months after inoculation with this strain. The peach, daisy, apricot, gooseberry, pecan, honeysuckle, and some other strains show infections on the daisy within a week and sometimes less after inoculation. The hop strain will not produce a tumor on the daisy, although the daisy strain will produce a tumor rather readily on the hop root or stem.

The apple strain isolated from the hairy root illustrated in Figure 1 did not produce so many infections on its natural host as did the peach, hop, and daisy strains on theirs. These last three strains may produce 90 to 100 per cent infection. A weakly pathogenic colony of the apple strain may produce less than 20 per cent infection on apple trees, and an infectious colony 40 to 50 per cent. The same colony may cause 60 to 70 per cent infection (or even more) on Paris daisy and *Impatiens balsamina*. Reisolation colonies from apple-crown gall on daisy produce as high as 80 per cent infection. Ten infectious reisolation colonies obtained from roots on daisy (fig. 4, A*a*), when inoculated into 54 daisy stems, produced roots on 9 (fig. 7, A, B, D); tumors on 34, some with root primordia (fig. 7, C); and no infection on the remaining 11.

REACTIONS OF VARIOUS HOSTS TO INOCULATIONS

Young string-bean plants inoculated with the apple hairy-root isolation did not soon produce outgrowths, as four weeks or longer elapsed before definite swellings appeared. Bean plants of the same age inoculated at the same time with the hop strain developed 6-mm. tumors within three weeks. When bean stems were inoculated with the apple hairy-root isolation definite tumors rather than roots or a combination of roots and tumors were produced.

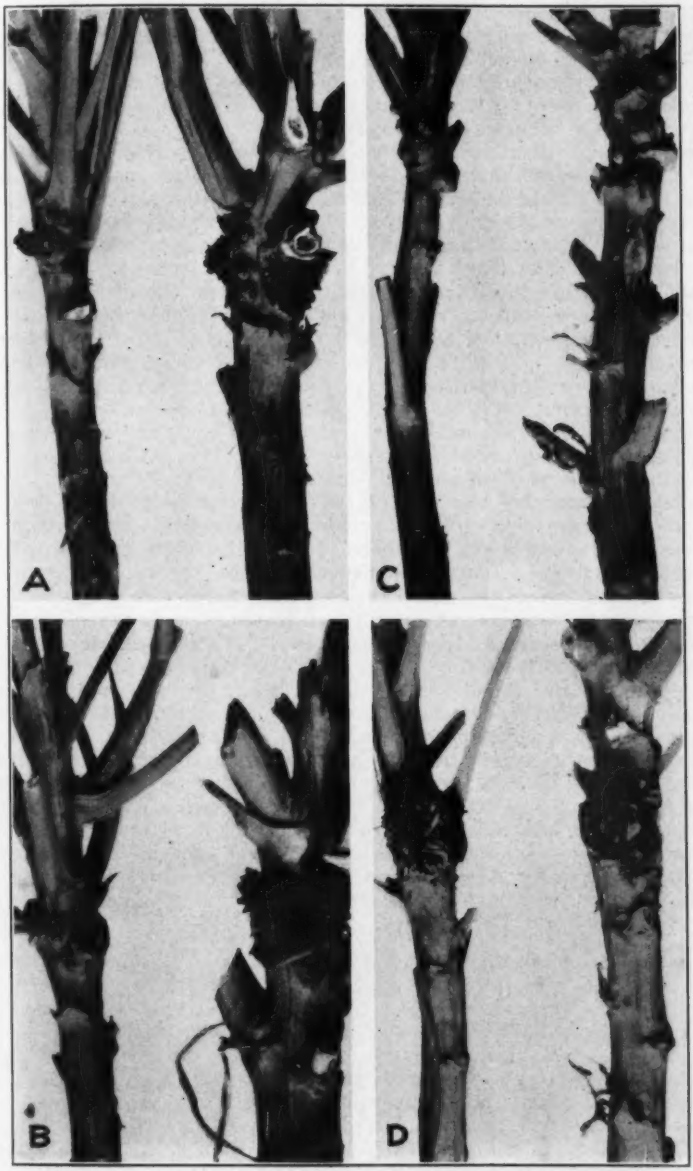

FIGURE 7.—A–D, Paris daisy stems inoculated, March 13, 1928, with four different colonies reisolated from outgrowths shown in Figure 4, A6. Photographed May 12, 1928. All natural size

When the rose was inoculated with the cultures used for beans and daisies it was slow to show infection, and when very tender shoots were inoculated infection did not appear for six weeks or longer. The type of outgrowth was a tumor with root primordia.

The castor-bean plant (*Ricinus communis*) was thought for some time to be immune to the apple strain. One set of inoculations appeared to be negative after two months. While another set of inoculations was in progress to check up this earlier one, small outgrowths appeared on the first set three months after inoculation, and on the second set between two and three months after inoculation. The tumors never grew larger than 4 mm. across, but they were definite infections which might easily have been overlooked if the controls had not been so definitely without swellings. The percentage of infection was about 50. Some of the Ricinus stalks were split longitudinally through the inoculated area, and little masses of tumors were found in the pith extending into the hollow center at these points. To make absolutely certain that the external and internal outgrowths were caused by the organism that had been inserted, platings were made from the tiny outside tumors and from the pith outgrowths, and crown-gall colonies appeared on both sets of plates. Subcultures made from colonies on these two sets of plates produced tumors when inoculated into daisies.

Ricinus is an acid plant with a pH of 5.4, but the peach and apple stems are more acid (pH 4.8 and 4.6, respectively), and the apple strain causes infection on them. Evidently there is some other feature of Ricinus than its hydrogen-ion concentration, perhaps a high titratable acidity, which makes it unfavorable as a host. The hop and peach strains, however, found Ricinus a very favorable host and produced tumors three-fourths to 1 inch in diameter in four weeks on vigorous plants.

Geranium and tobacco, like Ricinus, are favorable hosts to many strains of *Bacterium tumefaciens*, but are only slightly susceptible to the apple strain. For some time these plants were considered immune to the apple strain. Small flat inconspicuous outgrowths (4 to 5 mm. in diameter) of the tumor type were produced only under the most favorable conditions in two to four months, and but a small percentage of the inoculations caused infection (3 out of 24 with geranium and 4 out of 24 with tobacco). No root growths or tumors with roots were produced. Figure 8, D, shows the results of inoculating tobacco with the isolation from the hairy root shown in Figure 1.

DISTINCTIVE FEATURES

Isolations of the apple strain produce fewer virulent colonies, and the strain has a more limited number of host plants than any of the other strains except the rose. Slowness in producing tumors seems to be inherent in the strain itself, for slowness is characteristic of its action on its own host, the apple, as well as on other hosts. Moreover, the organism is slow in its growth in culture media. Like the rose strain, it is less translucent on beef and Thaxter (potato-dextrose) agar than other strains, which makes it appear in some stages quite unlike the crown-gall organism. In no medium used by the writer does it produce such a heavy growth as the other strains of *Bacterium tumefaciens*. Most isolations of the rose strain must be excepted, for this strain likewise failed to produce a heavy growth in the media used.

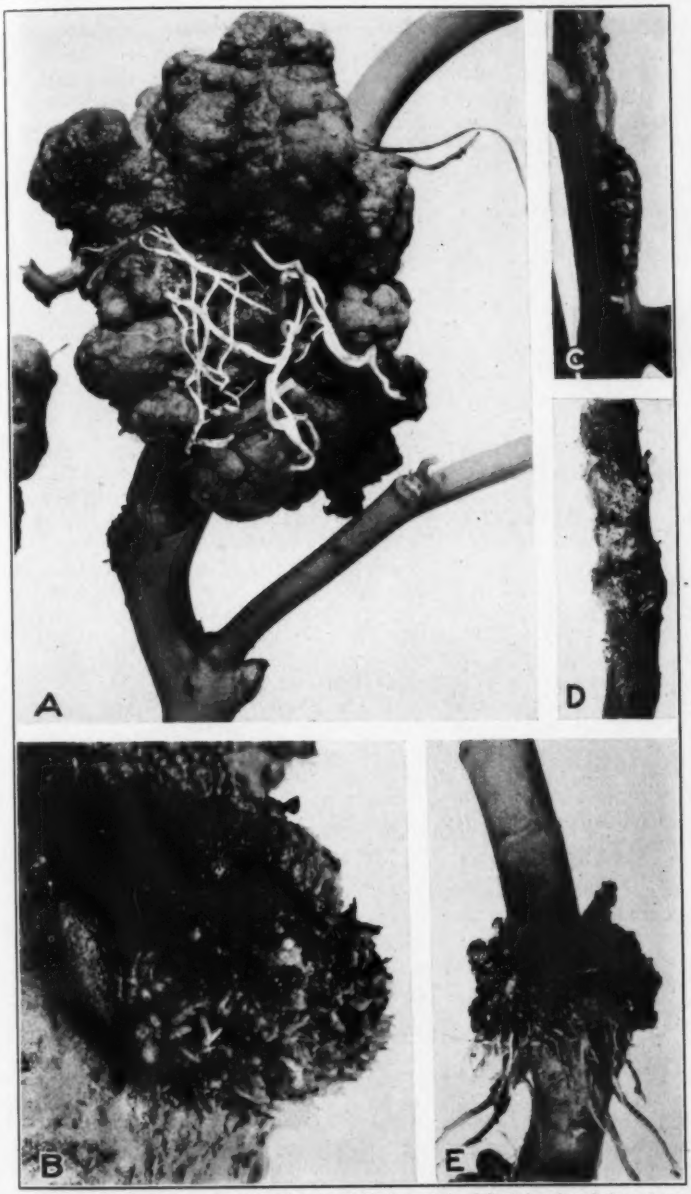

FIGURE 8.—A, Nasturtium stem inoculated with the hop strain of *Bacterium tumefaciens*, showing tumor and roots. Time, three months. Nearly natural size. B, Collards stem inoculated with poplar strain, showing tumor and roots. Time, two months. × 2. C, Tomato stem inoculated with daisy strain, showing tumors with a few root primordia. Time, five weeks. Natural size. D, Tobacco stem inoculated with the apple strain isolated from the apple hairy root shown in Figure 1. Time, five months. Natural size. E, Geranium inoculated with chrysanthemum strain, showing tumor and roots. Time, one year. Natural size

The apple and rose strains are alike (1) in producing comparatively few virulent isolation colonies, (2) in their habit of growth and appearance in culture media, (3) in having a more limited number of host plants than the other strains, and (4) in their slowness to produce tumors, although some tumors eventually become as large as the average tumor produced by other strains.

That the crown-gall organism is able to adapt itself to new conditions was shown by the apple strain in its reisolation colonies. The reisolations were made from apple hairy root produced on daisies. (Fig. 4, A*a*.) When these reisolation colonies were inoculated into other daisy stems infection appeared sooner than in the original inoculations. While with the original organism it took nearly two months for the inoculations to show infection, with the reisolation colonies it took a few days less than one month. The tendency to produce roots as well as definite tumors was retained in the reisolation colonies. (Fig. 7, A–D.) Of the 10 colonies picked off and tested, all proved infectious to daisy stems, and 80 per cent of the 54 inoculations were positive.

The writer has never been able to produce a definite swelling on tomato plants with the apple strain, although she has made inoculations in top and base and in the neighborhood of adventitious roots and has used plants of various ages and isolations and reisolations from different sources. Thirty-six tomato plants inoculated with the isolation from the hairy root shown in Figure 1 gave negative results. Small inconspicuous swellings with smooth surfaces were produced on tomato stems inoculated with a reisolation from apple tumor on daisy. The tomato stem is a favorable host to the peach and hop strains, but only moderately so to the daisy strain. (Fig. 8, C.) Tobacco is a favorable host to the rose (fig. 4, B), peach (fig. 4, C), and hop strains. If the peach strain of *Bacterium tumefaciens* in the soil should pass through the apple crown or root and produce infection, an isolation from this apple-peach tumor would probably still be able to produce an infection on the tomato stem as the original organism does. In his work with teratomata Smith (*18*) used the hop strain largely, and he pictured many hop tumors on tobacco.

The cultural characteristics of most of the reisolation colonies changed. The reisolations grew more rapidly and more abundantly in culture media than the original isolations, and on beef and potato-dextrose agar they looked more like the vigorous peach and hop strains than like the original isolations.

Further proof of the adaptability of the crown-gall organism was shown by the reisolation of the apple strain from tumors on Ricinus. The inconspicuous outgrowths which took three months to form on Ricinus produced reisolation colonies which developed outgrowths on daisy stems more rapidly than the apple-strain reisolations made from the daisy itself. The Ricinus reisolations of apple strain when used for inoculations showed good infections in the daisy in two to three weeks, whereas it took the daisy reisolations (apple strain) almost four weeks. Furthermore, the reisolation of the apple strain from Ricinus, like the reisolation of the apple strain from daisy, produced colonies which grew much more vigorously in culture media than the original apple isolation colonies, to which they were related.

It might be mentioned here that the strain isolated from a natural tumor on the crown of a hop plant became much more virulent and made a heavier growth in culture media after it was isolated from a hop tumor induced on a sunflower plant.

ROOT PRODUCTION WITH OTHER STRAINS OF BACTERIUM TUMEFACIENS

Although the apple alone of all the crown-gall strains seems to possess the inherent ability to produce roots whether it enters the crown, root, or stem of the apple tree or some other susceptible plant, still there are other strains of *Bacterium tumefaciens* that will produce roots on certain plants and under certain conditions. These roots are usually in combination with tumors and are produced mostly on plants that can be propagated easily from cuttings.

The hop strain produces roots very readily on *Impatiens balsamina*, the common garden balsam. The roots grow on or near the tumor and may be from 5 to 25 mm. or more in length. (Fig. 9, B.) The peach and poplar strains also produce roots on *I. balsamina* in less time than it takes the apple strain to do so. (Figs. 9, A, and 10, A–C.) It is very likely that all the strains of *Bacterium tumefaciens* will produce roots on this susceptible host and that each will display some individuality in its root production, as is shown by the four strains pictured.

One month after the balsam stems were inoculated with the hop strain, roots 10 to 12 mm. long had been produced, and the tumors were 1 to 1.5 cm. in diameter, or even larger. The roots on the stems of the plants inoculated with the poplar strain were not so long, and those inoculated with the peach strain were much shorter, being only 3 to 4 mm. In one month the inoculations, made with the original isolation from the apple hairy root and with two reisolations, showed tiny tumor swellings 3 to 4 mm. across with root primordia but no root extensions. The photographs showing root extensions of apple strain were made two months after inoculation. The experiment was repeated, but the apple strain was less active and only a few roots extended from the tumors even after three months. The three other strains showed vigorous roots.

The masses of roots often produced at the crown of chrysanthemum plants and the abnormal number of roots on rooted cuttings of chrysanthemum have been found to be produced in some cases by *Bacterium tumefaciens*. These masses of roots are not beneficial to the plant, for the vigor and substances that should go into the production of leaves and flowers are used in the thickened masses of roots. The organism isolated from chrysanthemum roots produces both roots and tumors when inoculated into susceptible plants. An example of a tumor with roots on a geranium produced by inoculating it with the chrysanthemum strain is shown in Figure 8, E.

The hop strain inoculated into Bryophyllum and nasturtium stems can produce definite tumors with roots. (Figs. 5, A, and 8, A.) So also can the poplar strain inoculated into collards stems. (Figs 8, B.) Occasionally the daisy strain will produce a few roots from the tumors on daisy stems.

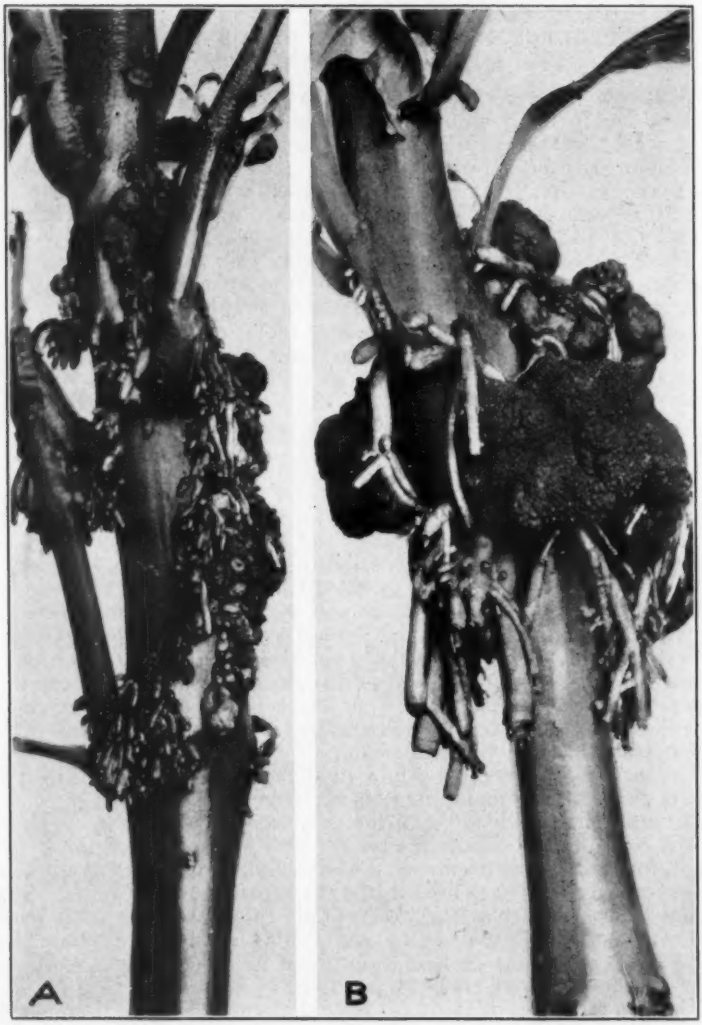

FIGURE 9.—*Impatiens balsamina* inoculated June 15, 1928: A, With peach strain of *Bacterium tumefaciens;* B, with hop strain. Photographed September 17, 1928. Both natural size

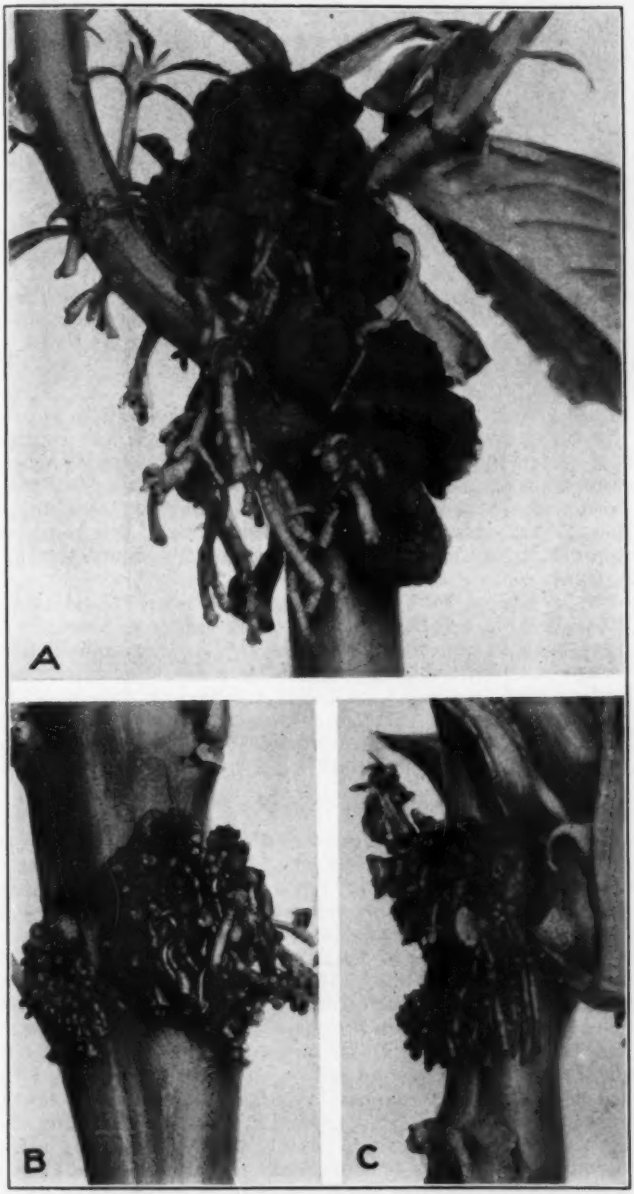

FIGURE 10.—*Impatiens balsamina* inoculated with different strains of *Bacterium tumefaciens*, June 15, 1928, and photographed September 17, 1928: A, Poplar strain; B, apple hairy-root strain; C, reisolated strain from apple hairy root. All natural size

STIMULATION OF ROOT PRIMORDIA TO ROOT FORMATION

Neither the rose nor the bean stems inoculated with the isolation from the apple hairy root produced roots in conjunction with the tumors (figs. 2, B*a*, B*b*, and C), but some of the tumor surfaces were roughened somewhat by root primordia. The bean tumors were stimulated to root production by chilling the whole plant in the refrigerator at a temperature of 10° to 14° C. for 20 hours, and then exposing it to a temperature of 25° to 27°. The primordia would extend into little roots 2 to 3 mm. long in four to eight hours. Some of the primordia that did not develop into roots in 24 hours could be made to do so by several sucessive changes from cold to warm temperatures. The change from cold to warm temperatures seemed to be necessary, as a pot of bean plants left in the refrigerator for 5 successive days did not show any root extensions from the three tumors until it had been in a warm room for about six hours. The tumors with smooth surfaces did not respond to the treatment, for roots did not appear.

The rose tumors with root primordia were stimulated to produce roots by being covered with sphagnum moss which was kept moist. Three of the eight tumors covered had produced roots when the sphagnum was removed after seven days. The stimulus here may have been a change of temperature also, for in a hot greenhouse evaporation must take place from the moist sphagnum; this could reduce the temperature of the tumor and might cause a stimulation to root development.

The roots induced by the ice-box and moss-ball stimulation were from 2 to 8 mm. in length. In some cases they were longer, but the tips dried back in the warm room as the root extended and true measurements could not be made, The roots of the rose tumors may have formed in a short time, but a week had elapsed when the moss ball was removed. One root from a rose tumor was 15 mm. long when uncovered, but the root was too fragile to survive the period required for photographing.

DISCUSSION

Can this tendency to root production of the crown-gall organism be an indication of a primitive soil form and the first manifestation of its pathogenicity? And, further, can it be that after passing through the apple or some other host and back into the soil the organism shows more active pathogenic features? And if so, why could not these more active features be manifested in the more definite tumor form instead of roots? This first act in pathogenicity might explain to some extent the slow adaptation of *Bacterium tumefaciens* to the apple as a host and the more frequent hairy-root type of crown gall which has been so confusing to nurserymen, and it might also explain the real tumor form of crown gall on apple to be produced by an organism beyond the first adaptation stage to the apple as host.

The organism reisolated from apple hairy root on daisy (fig. 4, A*a*) produces roots and tumors more rapidly than the original isolation, and it grows more rapidly and profusely in culture media than does its forebear. That the crown-gall organism in general is an adaptive one is shown by the many plants that it infects, for with the exception of the monocotyledons, outgrowths caused by it have been found on most of our common fruits, vegetables, and ornamental plants.

The fasciation of stems of various plants like the sweet pea and the nasturtium known to be caused by the crown-gall organism might be produced by the same soil pathogenic form demonstrating its pathogenicity at the crown of a plant by fasciation instead of root or tumor production. The writer found that the organism isolated from fasciated sweet-pea stems was a weakly pathogenic one which would infect tiny sweet-pea seedlings but not plants 5 or more inches tall. The percentage of infection in the seedlings was only 37.

It is not known why some isolations of the apple strain are weakly parasitic and others actively parasitic. It may be that the less active organism is the primitive crown-gall parasite, or that it has had to survive a long residence in the soil between the times of host residence, while the more active one has passed through and infected one or more apple trees in succession and so increased its virulence. Or it may be that those very active crown-gall strains such as peach, hop, and daisy have had still more passages through various hosts, each time becoming more adaptable and virulent before returning to the soil.

SUMMARY

The crown-gall organism (*Bacterium tumefaciens*) can stimulate plants to produce roots as well as tumors. The strain isolated from apple trees produces both roots and tumors. Both the hairy-root and the tumor types of infection produced by the apple strain are considered symptoms or manifestations of the same disease.

The apple strain is slower to infect susceptible hosts than other strains except the rose. It is also slow to infect the apple tree.

The Paris daisy and *Impatiens balsamina* respond to inoculations with the apple strain by producing masses of roots and small tumors; the rose and bean stems by producing definite tumors instead of masses of roots; *Bryophyllum pinnatum* by producing masses of roots and tumors without roots. Tobacco, geranium, and Ricinus are very indifferent hosts on which a few inconspicuous outgrowths without roots are produced in two to four months. The results on tomato were negative.

When first isolated the apple strain differs somewhat in appearance from the other crown-gall strains (the rose excepted) when grown in culture media. These differences disappear when the apple strain is passed through another host and reisolated. These reisolation colonies tend to produce infection in less time than the orginal colonies.

Like the other strains of *Bacterium tumefaciens*, the apple strain is an adaptable one (though less so than the other strains), and when it passes through another host than the apple it becomes more virulent.

Root production is not limited to the apple strain of *Bacterium tumefaciens*, for the peach, hop, poplar, chrysanthemum, and other strains also produce roots.

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THE DISTRIBUTION OF VITAMIN A IN SOME CORN-MILLING PRODUCTS[1]

By CLARA ROCKE MEYER, *Laboratory Assistant in Nutrition*, and ROSSLEENE ARNOLD HETLER, *Assistant Professor of Nutrition, Department of Home Economics, Agricultural Experiment Station, University of Illinois*[2]

INTRODUCTION

It was shown by Coward in 1923 (*1*)[3] that when whole yellow corn is used as the source of fat-soluble vitamins, large quantities are necessary to produce growth in rats which have been depleted of these vitamins. In more recent investigations Steenbock and Coward, (*2, 21*) have demonstrated that a much smaller quantity of whole yellow corn supplies sufficient vitamin A to produce a healthy condition in test animals if vitamin D is furnished in the basal ration. These results indicate, as suggested by the investigators, (1) that yellow corn is a remarkably poor source of vitamin D and that previous failure to promote growth on a low level of whole yellow corn was due to a deficiency of vitamin D rather than to a deficiency of vitamin A, and (2) that whole yellow corn is a rich source of vitamin A.

Millhouse and Croll,[4] using the hand-dissected structures of whole yellow corn, found that the fat-soluble vitamins of yellow maize are located in the pigmented endosperm of the kernel. Steenbock and Coward (*2, 21*), also using hand-dissected corn, could state with greater certainty that vitamin A is concentrated in the endosperm of the yellow corn kernel, since in their study an irradiated basal diet furnished the antirachitic factor.

In the work here reported a qualitative and quantitative study of the distribution of vitamin A was made on all of the by-products recovered from the wet-milling process for the commercial separation of cornstarch from whole yellow corn.

At the time the milling products included in this study were obtained, yellow corn only was used by the milling company from which they were secured.[5] The chemist reported a recovery of the whole yellow corn from the wet-milling process as follows:

Milling product	Per cent
Starch	65. 0
Corn germs	7. 0
Corn-germ meal	3. 5
Crude corn oil	3. 4
Grits	3. 6
Gluten	10. 0
Steep water (dried)	5. 0
Bran (reel slop)	6. 8
Gluten feed	25. 4

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[2] Grateful acknowledgment is made to Hilda Croll Koser, under whose direction this study was begun.

[3] Reference is made by number (italic) to "Literature cited," p. 779.

[4] MILLHOUSE, L. E., and CROLL, H. Unpublished data.

[5] Thanks are extended to the Staley Corn Products Co., Decatur, Ill., for the milling products used in this study.

The wet-milling process is a well-known commercial procedure for the preparation of cornstarch. The process as described by Rolfe (*13*) does not differ except in unimportant and minor details

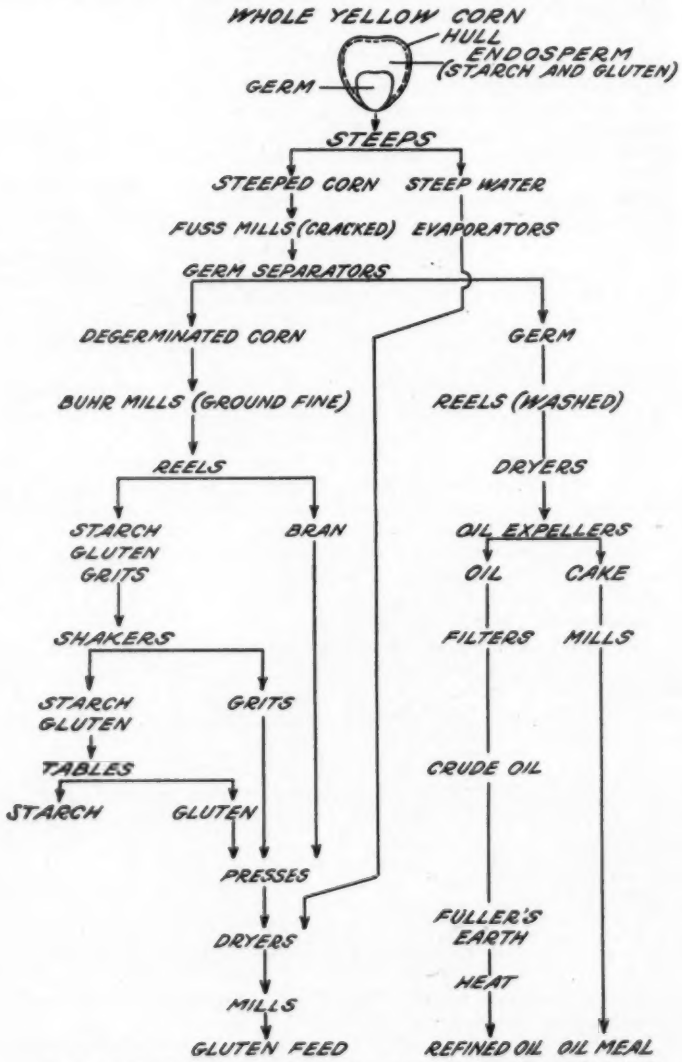

FIGURE 1.—Flow sheet indicating the relation of various corn-milling products to the structure of the corn kernel

from that employed by the milling company from which the products used in this work were obtained. It is almost entirely a physical rather than a chemical procedure involving comparatively

low temperatures and little chance for oxidation. The flow sheet in Figure 1 is a condensed diagram indicating the relation of the products here tested to the structure of the corn kernel and the general method of their separation by the wet-milling process.

EXPERIMENTAL PROCEDURE

Ophthalmia was developed in approximately 125 rats of the albino variety bred in the writers' own colony. Three series of animals were used in this study. In order to obtain rats of standard depletion in vitamin A, as Smith and Chick (*19*), Sherman and Storms (*18*), and other workers have found necessary, the mothers were given a diet low in this vitamin. For several weeks prior to and during breeding, and throughout the lactation period, a diet, essentially that used by Sherman (*15*, *16*, *18*), was fed. The diet consisted of two-thirds ground whole wheat and one-third whole-milk powder, with the addition of sodium chloride in the proportion of 1 per cent and meat scrap in the proportion of 5 per cent of the total weight of the wheat and

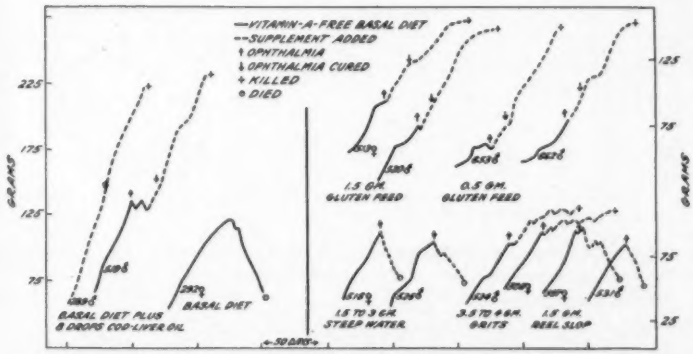

FIGURE 2.—Weight curves of rats receiving vitamin-A-free diets supplemented by various corn-milling products. When the vitamin-A-free basal diet was supplemented by 1.5 or 0.5 gm. of gluten feed daily, ophthalmia was cured; when the supplement was grits, ophthalmia was not cured and growth was very slow; when steep water or reel slop was used, ophthalmia was not cured and death resulted

milk powder. On the second or third day after birth the litters were reduced to eight young each. The experimental period was started when the young were 28 to 30 days of age.

The usual methods for individual caging, feeding, and caring for experimental animals were employed. Raised-bottom cages were used. The rats were weighed once a week for the first three or four weeks and then two or three times a week after the development of ophthalmia and during the succeeding test period. The food intake was carefully noted at the time the animal was weighed and whenever a new supply of food was given. In order to reduce the error incurred by the scattering of food, 500 c. c. porcelain jars with screw tops, having an opening about 1½ inches in diameter, were used. The jars were filled about one-third full of food.

An attempt was made to determine the standard rate of growth for recovery of animals in series 2 and 3 by feeding cod-liver oil, 8 drops daily, after the appearance of ophthalmia. (Table 1 and fig. 2.) This is probably a better method by which to judge the extent of the

vitamin deficiency than the uninterrupted growth of an animal fed cod-liver oil from the beginning of the experimental period (*9*). (Table 2.) A "negative control" animal was maintained in each litter on the vitamin-A-free basal diet (Sherman, *17*). (Table 3.)

TABLE 1.—*The effect of cod-liver oil on ophthalmia and growth of vitamin-A-deficient rats*

[Positive controls]

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Amount of supplement daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test period[a]	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
		Days	*Gm.*		*Gm.*		*Gms.*	*Drops*	*Gm.*	*Gm.*	*Weeks*	*Days*	*Gm.*
519♂	6	31	56	27	128	7	142	8	61.0	74.0	6	114	231
645♀	10	24	40	34	45	11	82	8	19.0	41.0	7	119	157
671♂	13	30	34	28	71	9	119	8	38.6	69.0	5	111	165
658♀	12	29	44	31	78	9	119	8	42.2	63.0	5	111	162
817♀	17	29	55	30	158	6	176	8	65.5	83.0	3	79	205
821♂	17	29	64	34	164	7	186	8	68.2	68.5	4	79	220
832♂	14	29	53	38	168	10	206	8	65.6	67.7	4	83	228
839♀	15	29	46	28	120	12	158	8	55.5	50.6	4	83	179

[a] The animals were all killed at the end of the test period.

TABLE 2.—*The prevention of ophthalmia and the growth of rats fed a vitamin-A-free basal diet supplemented by cod-liver oil*

[Positive controls]

No. and sex of rat	Litter No.	Initial age and weight		Amount of supplement fed daily	Average weekly food intake (vitamin-A free basal diet)	Duration of test period [a]	Final age	Final weight
		Days	*Gm.*	*Drops*	*Gm.*	*Weeks*	*Days*	*Gm.*
305♀	3	29	46	8	58.0	8	85	163
297♂	2	29	44	8	47.4	12	113	188
289♂	1	28	43	8	63.0	8	84	210
535♂	8	26	40	8	59.0	7	74	170
511♀	5	32	46	8	51.2	7	81	144
655♀	11	29	36	8	45.9	10½	103	158
672♂	13	30	23	8	56.4	10	101	165
840♀	15	29	39	8	60.1	8	83	165

[a] The animals were all killed at the end of the test period.

TABLE 3.—*The effect of vitamin-A-free basal diet only on ophthalmia and growth of vitamin-A-deficient rats*

[Negative controls]

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of death from ophthalmia and weight of animal		Average weekly food (vitamin-A-free basal diet)		Duration of test period after incidence of ophthalmia [a]	Final age
				Days after experiment began	Body weight	Days after development of ophthalmia	Weight at death	Before development of ophthalmia	After development of ophthalmia		
		Days	*Gm.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Weeks*	*Days*
308♂	3	29	45	35	100	8	89	29.2	26.0	1	72
298♂	2	29	48	35	100	47	62	36.0	31.0	7	111
292♀	1	28	42	28	106	45	70	48.0	35.6	6	101
322♀	4	29	40	37	120	42	70	55.4	29.0	6	108
518♀	6	31	48	27	101	23	78	54.2	44.0	3	81
523♀	7	26	36	29	73	21	52	38.7	29.0	3	76
504♂	5	32	50	34	88	7	100	42.0	41.0	1	73
534♂	8	26	42	27	86	14	80	48.0	32.0	2	67
641♂	10	24	36	34	62	50	45	40.0	39.4	7	108
640♀	9	31	52	34	52	53	62	17.8	26.0	7	118
649♀	11	29	36	33	60	33	60	33.3	47.2	4	95
657♂	12	29	44	33	68	33	76	31.4	45.6	4	95
665♀	13	29	46	28	68	38	63	32.5	31.0	5	95
844♀	16	28	40	28	121	28	104	63.0	54.0	4	84
820♂	17	29	62	14	118	26	153	75.0	71.0	4	71
852♂	19	30	58	28	158	28	123	80.0	49.5	4	86
836♂	15	29	42	24	120	16	120	52.0	62.0	2	69
828♀	14	29	47	27	125	13	130	50.0	70.0	2	69

[a] The test period terminated with the death of the animal in each case.

A vitamin-A-free basal food was given the test animals, which they were allowed to eat ad libitum. The antirachitic factor was supplied in the food by irradiation in shallow agate pans at a distance of 20 inches from the lamp for one-half hour. The food was stirred at the end of each 15 minutes of irradiation (*21*, *20*). A Hanovia luxor mercury arc lamp was used.

The basal diet was composed of the ingredients in the percentage amounts given below:

	Per cent
Extracted casein	18
Osborne-Mendel salt mixture	4
Crisco [6]	20
Starch	57
Sodium chloride	1

The casein was purified by the alcohol-extraction method of Osborne and Mendel (*10*, *11*). It was then air-dried for several days and subsequently spread out in shallow pans and heated from 8 to 12 hours in gas ovens at 120° C. (Drummond (*3*, *6*, *7*).)

The basal food was mixed and stored in glass jars in a refrigerator. If it was to be used within a short time it was first irradiated and then stored in the refrigerator. A week's supply was usually irradiated at one time.

The water-soluble vitamin-B factors were supplied by dried brewers' yeast, five-tenths gram per animal daily, fed separately.

The development of definite ophthalmia was taken as a sign of vitamin-A deficiency. (Morgan (*9*), Steenbock and Coward (*21*),

[6] Drummond (*5*), Daniels and Laughlin (*4*).

Steenbock, Sell, and Buell (*22*).) With the depleted animals used, definite eye symptoms almost invariably preceded cessation of growth and developed from 25 to 35 days after the experimental period began.

As soon as the eye symptoms became persistent, the test food was added to the diet. It was given separately in definitely weighed portions, or later, especially in the case of foods not entirely eaten when fed separately, as a definite part of the basal diet. The supplement replaced the same percentage of starch, or Crisco, or parts of both the starch and the Crisco; when thus mixed with the basal food, the test material was added after the irradiation of the purified ingredients. (Willimott and Wokee (*23*).) When fed separately the daily portion of the test food was moistened with distilled water immediately before feeding, for all of the foods, with the exception of he oils, were extremely dry and powdery, and the moistening reduced possible loss by air drafts.

The vitamin-A value of the test food was judged chiefly by the minimal dosage necessary for the ultimate cure of the eyes. (Steenbock and Coward (*21*).) A resumption of body growth was also observed.

EXPERIMENTAL RESULTS

In order to make this investigation quantitative in nature, it was necessary first to conduct a preliminary qualitative study of the corn-milling products. Each of the products was fed in accurately weighed amounts of 1.5 gm. daily to at least two rats in which opthalmia had developed. The results obtained in this first series of animals as indicated by the ultimate positive or negative cure of ophthalmia were without exception as shown in Table 4.

TABLE 4.—*The effect on ophthalmia of various corn-milling products when used as supplements to a vitamin-A-free diet*

Supplement	Amount fed daily per animal	Number of rats	Effect on ophthalmia	Effect on growth
	Grams			
Steep water	1.5	3	Not cured	Decline and death.
	2.3	1	do	Do.
	3.0	1	do	Do.
Reel slop	1.5	3	do	Do.
	1.8	1	do	Do.
	2.3	1	do	Do.
Grits	1.5	2	do	Very slow decline; all but 1 lived.
	2.6	1	do	
	3.4	1	do	
	4.0	1	do	
Gluten	1.5	2	Rapid cure	Normal growth.
	1.0	5	do	Do.
	.5	2	do	Do.
	.25	3	do	Do.
Gluten feed	.1	3	Not cured	Growth finally arrested; 1 died.
	1.5	8	Rapid cure	Normal growth.
Germs	1.5	3	Not cured	Slowly declined; 1 died.
	2.0	4	do	All 4 died.
Germ meal	1.5	2	do	Rapid decline; death.
	3.0	2	do	Do.
Crude corn oil	1.5 to 1.7	5	Slow cure	Lived and grew.
	.9 to 1.2	6	do	Do.
	.28 to .4	3	Not cured	All died.
Refined corn oil	1.5	2	do	Decline and death.
	1.0	1	do	Do.
	.6	1	do	Do.
Whole yellow corn	1.5	5	Rapid cure	Normal growth.
	1.0	8	More slowly cured	Do.
	.75	5	Never permanently cured.	Growth approaching normal.
	.5	5	do	Growth arrested; 3 died.
	.25	5	Not cured	Low rate of growth; all died.

In the case of steep water, reel slop, and grits, which were more or less poorly consumed as separate fractions, an attempt was made to feed at a higher level in order to test for traces of the vitamin. These three by-products were fed, therefore, as 50 per cent of the diet, replacing the same percentage of starch in the basal ration. As a result, the daily dosage was increased as shown in Table 4. In each case, as the table shows, despite the higher level reached in the feeding of the supplement, there was neither a cure of opthalmia nor a resumption of growth. The animals fed the steep water exhibited extremely sore eyes, with considerable pus and hemorrhage, and in some instances complete atrophy of the eyeball. The feces were watery and had the characteristic brown color of the steep water. The animals receiving the reel slop or bran supplement developed extreme ophthalmia to the same extent, apparently, as those on the steep water. The feces, however, were very large, due, probably, to the great extent of indigestible crude fiber in the reel slop. The grits supplement was better consumed than the steep water or the reel slop. There was a slow development of extreme ophthalmia in the case of animals on this fraction, but at no time were the eyes cured. It is apparent from these results, that the three by-products named above are almost if not totally lacking in vitamin A. (Fig. 2.)

Gluten feed in 1.5-gm. daily amounts produced a rapid cure of ophthalmia in eight rats. (Table 4.) The gluten feed is composed of reel slop, grits, steep water, and gluten. (Fig. 1.) Since all these products except gluten when tested separately were almost if not totally lacking in vitamin A, it seemed necessary only to test the gluten in a quantitative manner for this vitamin. The various levels of feeding used and the results obtained are shown in Table 5.

TABLE 5.—*The effect of feeding corn gluten on ophthalmia and growth of vitamin-A-deficient rats*

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Ophthalmia not cured		Amount of supplement fed daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test period*	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement to diet	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
		Days	*Gm.*		*Gm.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Weeks*	*Days*	*Gm.*
306♂	3	29	46	39	98	10	110	.....	.....	1.50	41.6	44.8	8	120	170
294♀	1	28	43	28	113	14	144	.....	.....	1.50	35.0	54.3	7	112	176
521♂	7	26	31	29	70	10	93	.....	.....	1.00	45.5	44.8	8	109	138
529♂	8	26	42	29	89	10	102	.....	.....	1.00	56.5	55.5	8	109	182
533♀	8	26	38	27	74	10	84	.....	.....	1.00	39.2	48.4	8	109	152
508♀	5	32	50	32	106	6	112	.....	.....	1.00	58.0	59.0	7	115	168
515♂	6	31	50	32	114	6	123	.....	.....	1.00	53.0	60.0	7	114	195
504♂	5	32	36	34	88	14	122	.....	.....	1.00	42.0	50.0	6	115	172
653♂	11	29	44	29	54	6	71	.....	.....	.50	33.5	44.1	8	117	160
663♂	12	29	46	33	75	14	108	.....	.....	.50	31.4	44.7	8	117	175
650♂	11	29	40	32	61	14	77	.....	.....	.25	29.3	37.1	8	117	136
660♂	12	29	44	33	83	9	102	.....	.....	.25	27.0	42.0	8	117	147
666♀	13	29	42	32	66	14	85	.....	.....	.25	36.8	40.0	8	117	122
661♂	12	29	43	33	72	.....	.....	34	95	.10	36.6	37.6	5	96	95
667♀	13	29	50	33	78	.....	.....	55	121	.10	43.1	37.2	8	117	121
651♂	11	29	37	33	58	.....	.....	55	93	.10	37.7	38.0	8	117	93

* All animals were killed except 661 which died with ophthalmia.

As previously stated, gluten is recovered in quantity equivalent to about 10 per cent of the whole corn. If then all the vitamin A of the whole yellow corn were concentrated in the gluten, similar results should be obtained with 0.1 gm. of gluten and 1 gm. of whole yellow corn fed daily. One gram of the whole yellow corn fed daily gave a comparatively rapid cure of ophthalmia and produced normal growth. It was found, however, that 0.1 gm. of gluten was not sufficient to cure or even to arrest ophthalmia. (Fig. 3 and Table 5.) One-fourth of a gram of gluten, however, cured the eye lesions, and, in addition, produced growth in the vitamin-A-depleted rats. Some of the vitamin, therefore, must have been lost in the separation of gluten from the corn or is present in other structures of the corn kernel.

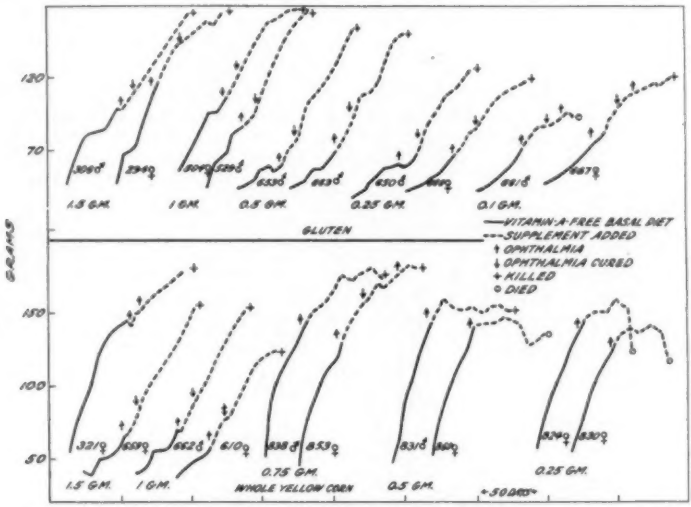

FIGURE 3.—Weight curves of rats receiving vitamin-A-free diets supplemented by various quantities of gluten and whole yellow corn

The results of the preliminary study indicated that some of the vitamin A of corn occurs in the germ structure of the kernel. A cure of ophthalmia was demonstrated with the crude corn oil. The germ itself, however, and the germ meal at the levels fed did not cure the eyes. The animals barely maintained weight and eventually died. The animals given the germ fared better than those given the germ meal. (Fig. 4.) But since a daily intake of 2 gm. of the germ was followed at best by only a slight improvement in the eye condition (Table 4), it was concluded that the small amount of vitamin A present in the germ had to be pressed out in the germ oil before its presence could be detected. The results of the quantitative study of crude corn oil, which are summarized in Table 6, show that crude corn oil is comparatively rich in vitamin A. (Fig. 4.)

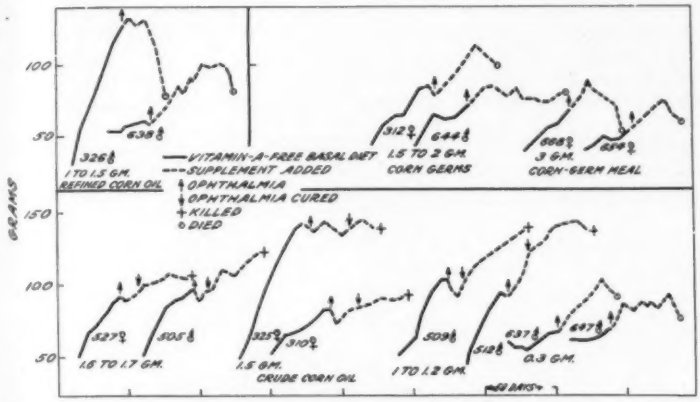

FIGURE 4.—Weight curves of rats receiving vitamin-A-free diets supplemented with various corn-milling products. When the vitamin-A-free basal diet was supplemented with one or more grams daily of crude corn oil, ophthalmia was cured and the animals lived; when the supplement was corn germs, corn-germ meal, or refined corn oil, ophthalmia was not cured and death resulted

TABLE 6.—*The effect of crude corn oil on ophthalmia and growth of vitamin-A-deficient rats*

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Ophthalmia not cured		Amount of supplement fed daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test periods [a]	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
		Days	*Gm.*		*Gm.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Weeks*	*Days*	*Gm.*
310♂	3	29	43	38	80	22	82	.....	.....	1.5	35.0	17.5	8	120	90
325♂	4	29	49	48	138	28	140	.....	.....	1.5	46.0	31.0	6	119	140
514♂	6	31	52	29	92	12	98	.....	.....	1.55	45.5	16.0	8	114	124
505♂	5	32	47	34	98	14	96	.....	.....	1.66	45.0	17.0	8	120	124
527♀	7	26	34	29	84	12	92	.....	.....	1.7	48.0	19.0	7	109	106
525♀	7	26	36	32	82	9	100	.....	.....	1.0	41.0	31.0	7	109	106
509♂	5	32	53	29	105	12	100	.....	.....	1.0	51.0	40.0	8	115	138
512♂	6	31	50	29	88	12	122	.....	.....	1.2	52.0	44.4	8	114	136
642♂	10	24	57	34	58	27	93	.....	.....	1.0	34.4	28.0	9	120	132
634♂	9	31	45	38	47	40	102	.....	.....	.9	25.6	26.7	8	127	124
643♂	10	24	33	34	54	30	86	.....	.....	1.0	35.0	34.4	9	120	118
646♀	10	24	35	34	58	.....	.....	46	74	.4	36.6	25.2	6½	104	74
637♂	9	31	55	38	64	.....	.....	52	87	.28	23.0	17.5	7½	111	87
647♂	10	24	35	34	38	.....	.....	51	57	.3	14.0	19.2	7½	109	57

[a] All animals were killed except 646, 637, and 647, which died with ophthalmia.

Refined corn oil was not only incorporated in the basal diet to replace the 20 per cent Crisco, but was also fed separately in daily fractions. From the results shown in Table 4 and Figure 4 it is evident that there is little if any vitamin A in refined corn oil. It may be concluded that there is sufficient heat under oxidative conditions involved in the refining process to destroy all of the vitamin A that may have been present in the crude corn oil.

A quantitative study of the unmilled yellow corn was undertaken. The results obtained (Table 7) show that 1 gm. daily, or approximately

11 per cent, is the minimum requirement for permanent cure of ophthalmia and normal growth. Temporary cure of the eyes was obtained with 0.75 gm. daily, or approximately 9 per cent, and even with as little as 0.5 gm. daily, but the eyes again became extremely sore, a general decline occurred, and death resulted in most cases.

TABLE 7.—*The effect of (ground) whole yellow corn on ophthalmia and growth of vitamin-A-deficient rats*

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Ophthalmia not cured		Amount of supplement fed daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test periods	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement to diet	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
		Days	*Gm.*		*Gm.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Weeks*	*Days*	*Gm.*
321♂	4	29	54	44	143	5	154	.....	.....	1.50	60.3	50.7	5.5	112	180
307♂	3	29	47	39	98	10	110	.....	.....	1.50	39.1	46.0	7.5	120	176
304♀	2	29	50	35	90	11	95	.....	.....	1.50	33.2	40.4	8	120	178
659♂	12	29	42	33	68	6	79	.....	.....	1.50	28.8	39.0	8	117	153
652♀	11	29	48	33	66	9	82	.....	.....	1.50	25.6	43.0	8	117	155
656♂	11	29	44	33	62	7	89	.....	.....	1.0	27.6	47.0	8	117	178
662♂	11	29	42	41	70	7	91	.....	.....	1.0	27.0	43.7	8	117	167
664♂	12	29	42	31	74	11	96	.....	.....	1.0	29.2	47.5	8	117	162
669♂	13	29	36	33	68	7	76	.....	.....	1.0	26.2	43.3	8	117	148
670♀	13	30	32	30	58	9	78	.....	.....	1.0	46.7	45.4	8	117	135
829♀	14	29	49	28	133	12	147	.....	.....	1.0	66.0	71.5	8	113	170
837♀	15	29	44	28	123	12	132	.....	.....	1.0	63.5	74.6	8	113	167
845♀	16	28	51	28	133	18	147	.....	.....	1.0	59.2	66.7	8	110	178
822♂	17	29	63	30	170	.....	.....	56	198	.75	78.0	48.0	8	115	198
838♂	15	29	48	28	140	.....	.....	56	174	.75	67.7	49.1	8	113	174
846♀	18	28	39	28	114	.....	.....	46	109	.75	58.0	44.3	7	102	109
853♀	19	30	51	28	136	.....	.....	54	181	.75	70.5	72.0	8	112	181
856♀	19	30	53	28	134	.....	.....	54	167	.75	60.0	52.0	8	112	167
823♀	17	29	60	30	149	.....	.....	56	168	.50	70.2	75.2	8	115	168
831♂	14	29	54	28	147	.....	.....	56	147	.50	63.2	44.6	8	113	147
847♂	18	28	57	34	156	.....	.....	41	153	.50	52.4	57.1	6	115	153
854♂	19	30	51	32	157	.....	.....	55	158	.50	50.1	50.0	8	117	158
860♀	20	30	51	32	145	.....	.....	48	138	.50	60.0	49.0	7	110	138
824♀	17	29	58	30	142	.....	.....	37	128	.25	70.5	74.4	5	96	128
830♀	14	29	54	28	128	.....	.....	37	116	.25	57.0	61.4	5	94	116
848♀	18	28	40	36	127	.....	.....	20	103	.25	61.6	47.3	3	84	103
855♀	19	30	46	29	127	.....	.....	35	133	.25	59.8	67.4	5	94	133
861♂	20	30	56	34	141	.....	.....	29	140	.25	60.2	66.2	4	93	140

[1] Animals 846, 847, 854, 860, 824, 830, 848, 855, and 861 died; the rest were killed.

As a result of this study a flow sheet similar to that in Figure 1 may be drawn to indicate the distribution of vitamin A in the same milling products. Such a sheet is shown in Figure 5.

The condition of typical animals while on a diet deficient in vitamin A and after recovery from the effects of vitamin-A depletion is shown in Figure 6.

THE RELATION BETWEEN VITAMIN-A-CONTENT AND YELLOW PIGMENTATION

The high concentration of vitamin A in highly pigmented products of yellow corn, gluten, and crude corn oil as found in this study demonstrates the association of vitamin A with yellow pigmentation. Coward (*1*), in 1923, stated that some lipochrome, generally carotin, is always associated with the vitamin A in plant tissues and that where the carotin is found, particularly carotin exposed to sunlight,

there also the vitamin may be expected to be present. However, various other workers, as for example, Palmer and Kennedy (*12*) in 1921, Steenbock, Sell, and Buell (*22*) in 1921, and Drummond and Coward (*7*) and Rosenheim and Drummond (*14*) in 1920, have noted exceptions in the association of vitamin A with yellow pigmentation.

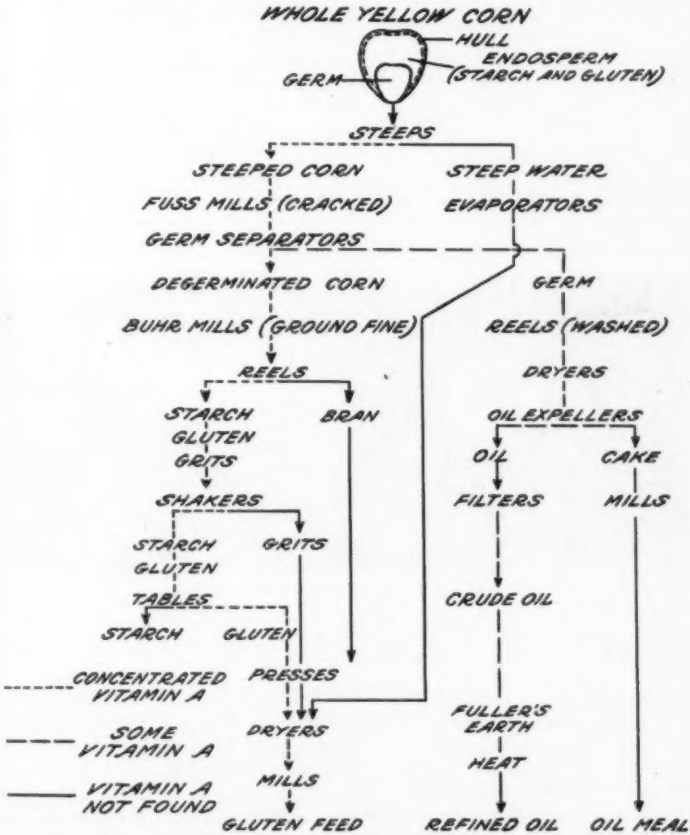

FIGURE 5.—Flow sheet indicating the distribution of vitamin A in various corn-milling products

Hauge and Trost (*8*), in a recent inheritance study of the association of vitamin A and yellow endosperm kernel, concluded that any genetic relationship existing between these two factors must be one of close linkage. They found that vitamin A was transmitted exclusively with yellow endosperm through the process of crossing and segregation.

SUMMARY

The rate of growth, and the recovery or nonrecovery from ophthalmia of vitamin-A-depleted rats were observed when their vitamin-A-deficient basal diets were supplemented with whole yellow corn and with various corn-milling products.

Whole yellow corn was found to be rich in vitamin A. Rapid growth was obtained when amounts of 1.5 gm. per animal were fed daily. The minimal amount needed to cure ophthalmia was 1 gm. daily, or approximately 11 per cent of the total food intake.

Gluten feed administered in amounts of 1.5 gm. daily rapidly cured the ophthalmic eyes of vitamin-A-depleted rats. Three of the four milling products that constitute the gluten feed—namely, steep water, reel slop, and grits—failed in every instance to effect a cure; gluten, the fourth constituent, was found to be extremely potent. The minimum amount of gluten required to cure ophthalmia was 0.25 gm. fed daily, and this amount also produced normal growth.

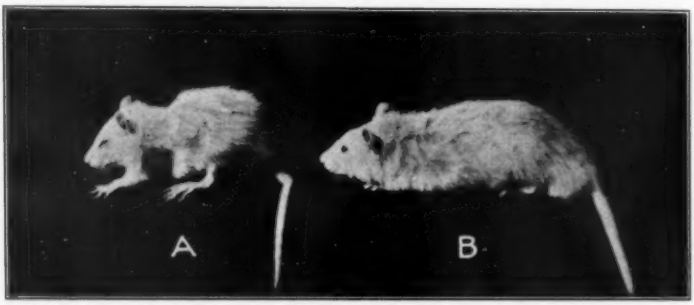

FIGURE 6.—Typical animals representing the vitamin-A-deficient rat, and the rat which has recovered from vitamin-A deficiency. A.—In the case of rat 531♂ ophthalmia was not arrested and a decline in growth resulted when the diet consisted of steep water, grits, reel slop, corn germs, corn-germ meal, and refined corn oil as sole sources of vitamin A. B.—When ophthalmic animals were fed yellow corn, gluten, gluten feed, or crude corn oil, the ophthalmia was cured and there was a resumption of growth, the animals finally appearing like rat 519♂

This finding shows that vitamin A is concentrated in the endosperm of the yellow-maize kernel. It furthermore unquestionably indicates that the vitamin-A concentration is greatest in that part of the endosperm lying next to the seed coats—the pigmented, nitrogenous outer layer of the endosperm.

Crude corn oil was found to cure ophthalmic rats when administered in daily doses of 1.5 gm. Corn germs, the source of the crude corn oil, were found to be so devoid of vitamin A that no cure could be obtained even when they were fed at a level of 2 gm. daily. The germ meal was found totally lacking in vitamin A. It was concluded that the small amount of vitamin A not found in the gluten is concentrated in or associated with the corn oil of the yellow corn germ and that it follows the oil in its removal from the germ. Refined corn oil was found to be totally lacking in antiophthalmic qualities and hence in vitamin A.

A high vitamin-A content was found associated with yellow pigmentation.

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THE INHERITANCE OF RHODE ISLAND RED CHICK DOWN-COLOR VARIATIONS AND THEIR RELATION TO COLOR VARIATIONS IN ADULT PLUMAGE [1]

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INTRODUCTION

Any inheritance studies involving variations in the down of chicks have a special theoretical interest because they fall within the category of larval characteristics. Relatively few distinctly larval characters have been made the subject of genetic studies, yet the bearing of these characters upon the problem of the gene in its relation to development is a fundamental one. Color patterns of chick down may be considered as in the nature of larval characteristics for the reason that in many cases the pattern appears to bear little relation to that of the adult. It is true that each adult plumage pattern has a definite corresponding down pattern, but frequently the two patterns have little in common. Both adult and chick down patterns vary to a certain degree, but very little attention has been given to how variations of one affect the expression of the other.

In addition to its theoretical bearing this study has certain practical aspects since any information of predicative value to be obtained from the chicks regarding the color qualities of adults is of considerable value. Rhode Island Red chicks vary widely in the shade of the red color. The lightest individuals are cream colored with only a tinge of red while the darkest are a chocolate brown.

MATERIALS AND METHODS

The stock used in this study was the Single Comb Rhode Island Red flock of the Kansas Agricultural Experiment Station. The strain had been bred for several years as a production flock, and an effort has been made to maintain standard qualities. The average color of the flock is a good dark red, probably above the average quality of most production-bred flocks. As in most flocks of this breed, there is considerable individual variation in the shade of the red color.

In order to keep the classification of chick down color as uniform as possible throughout the study, a standard series of skins was prepared. The skins were arranged in a Riker specimen mount and given grade numbers ranging from 1 to 5. These grades of down color are shown in Plate 1. As soon as chicks were removed from the incubator they were wing-banded and graded according to the standard series. Lighting conditions were kept as uniform as possible while grading was being done.

For recording variations in adult color a detailed description was made at 6 months of age. Wherever possible, standards similar to

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the one used for down color were made up. For recording the shade of red of surface and under color of plumage a Riker mount standard was used. This was made by taking sections of plumage from individuals showing the existing range of color. The record was made by entering the number of the standard grade which most nearly matched the color of the individual. The eye color recorded was that of the iris. The variations noted were in the shade of red which was determined by comparison with a prepared color standard. The standard series used for adult surface and under color and for eye color are shown in Plate 2. Variations in the amount of black pigment in the primary and secondary wing feathers were

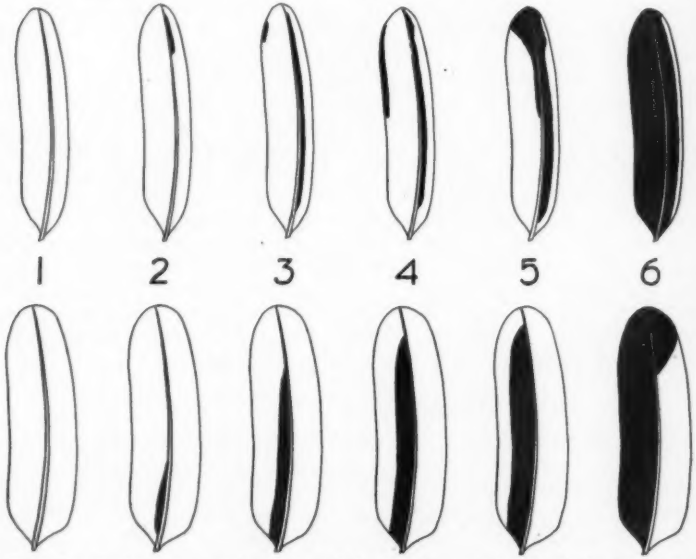
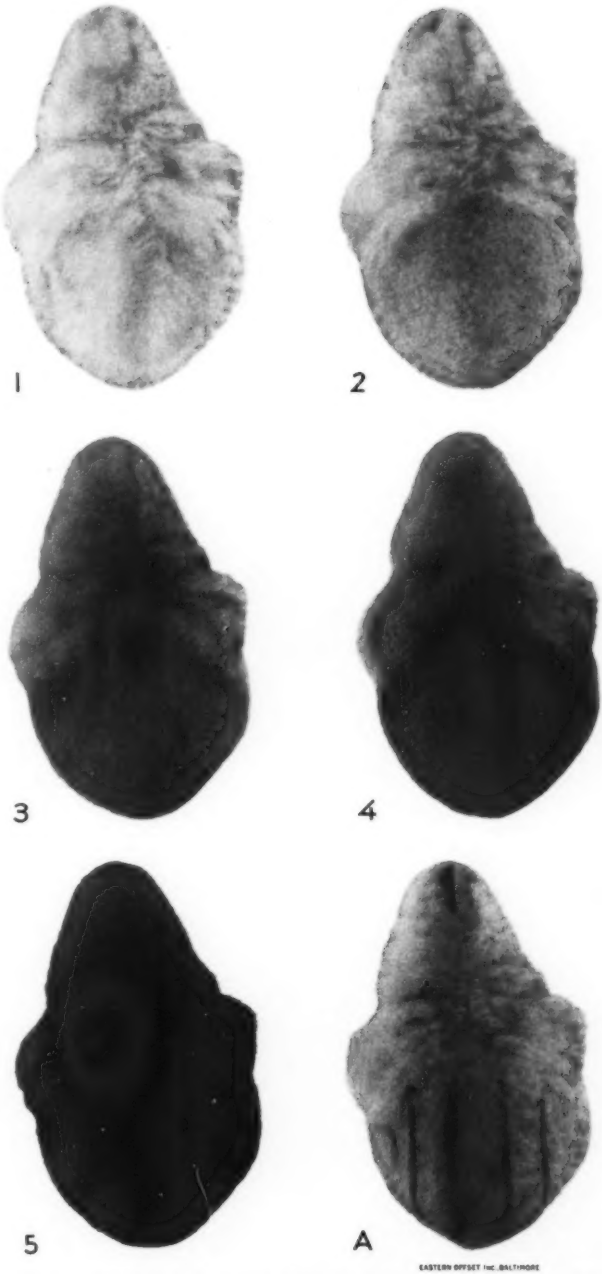


FIGURE 1.—Grade-number standards for variations in the amount of black pigment in the primary (upper series) and secondary (lower series) wing feathers of adult Rhode Island Red chickens

recorded by grade number given in a standard (fig. 1) showing the range of variations. The flight feathers used for recording the grade of black were the second full-length primary and the second secondary. In the description of the surface color the back, hackle, wing bow, and breast were graded separately. The occurrence of black pigment in any section of the surface was also recorded. In addition to recording the shade of under color, note was also made of the presence of smut [2] or of white in this portion of the plumage.

Because of the large number of birds involved it was impractical to preserve entire skins, but a fairly satisfactory substitute was devised. This was a feather card upon which sample feathers from seven representative sections of the plumage were mounted. The

[2] In the use of the term "smut" the writer has accepted the terminology of the poultry fancier. However, since this word has a different connotation in ordinary usage, the word "soot" would appear to describe much better the presence of black pigment in the under surface of the plumage.



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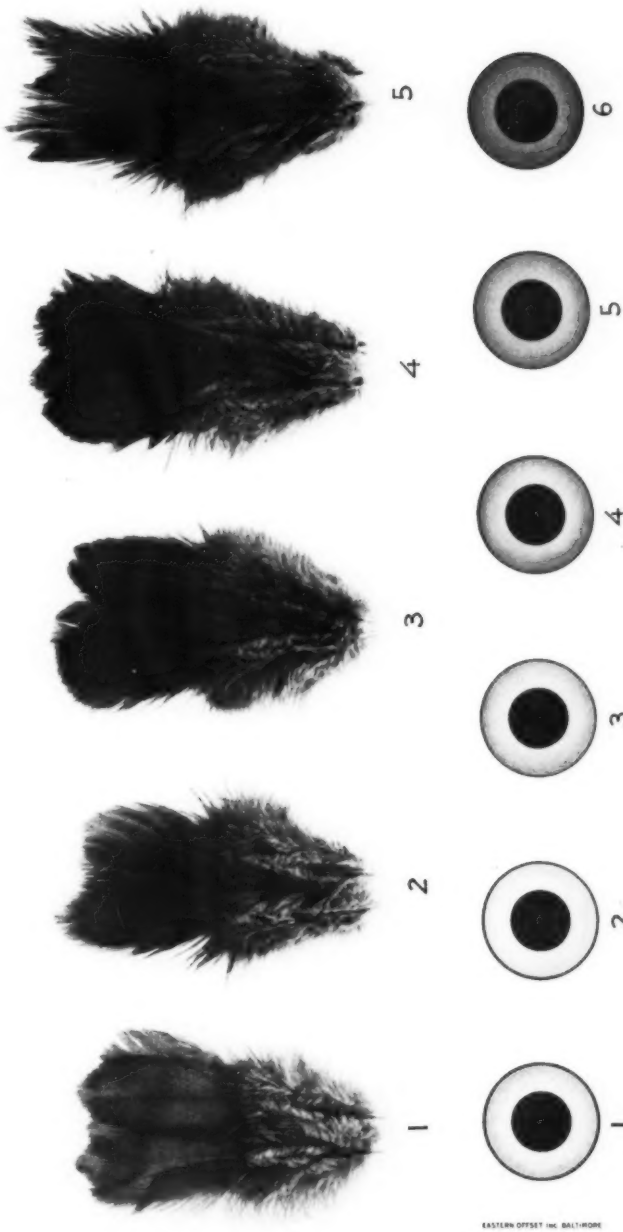
Chicks 1 to 5 show the standard series used for grading down color. Chick A shows striping in an extreme degree



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Standard color series used to grade adult surface and under color, and adult eye color. The tips of the feathers show the five grades of surface color. Feathers of the first four grades were taken from females but the grade 5 sample is from male plumage, since females seldom show the darkest color. Feathers 1 to 4 also show the four grades of under color

regions represented were the saddle, hackle, wing bow, back, breast, and the primary and secondary wing feathers. For mounting the feathers a 8 by 5 inch card bearing a double row of loosely set wire staples was used. The feathers were slipped beneath the staples, after which the staples were clamped tightly by the use of a long-lipped pair of pliers. This made for each individual a permanent record, and these records were arranged by families in standard filing cases. Figure 2 shows a filled feather card.

The work upon inheritance of down color covers the period from 1924 to 1928, but the data upon relation of down to adult color are only for the breeding seasons of 1927 and 1928. Relationships of down and adult colors were recorded for the entire period, but only

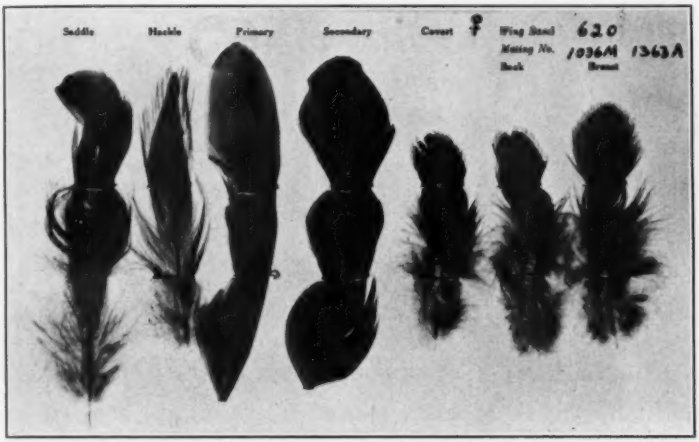

FIGURE 2.—A typical filled feather card containing sample feathers from seven representative sections of the plumage of a Rhode Island Red chicken

during the last two years were the classifications made from standards sufficiently definite to provide data of much value.

INHERITANCE OF DOWN COLOR

During the hatching seasons of 1925 to 1928 selections were carried out for the establishment of a light and dark down strain. Previous to the first season's matings no definite standard had been devised and the birds used for initiating the two strains had merely been described as individuals lighter or darker than the average. During the 1925 hatching season the chick down standard was first used; so that the matings beginning with 1926 were of birds of a known down grade. For the light strain, only birds whose down colors were of grades 1 and 2 were used, most of them being of the lighter grade. In the dark down selections all individuals used were of grades 4 and 5. Table 1 shows the results of the four years' selection. It will be seen that there is no sexual dimorphism with respect to down color, since the males and females in each generation show very similar distributions. Selection was much more effective in the light strain than in the dark one, and during the 1928 season a large

majority of the chicks in the light strain were of grade 1 with a limited number falling in grade 2. The last two years' results for the dark strain show a higher percentage of darker chicks than for the two preceding years, but in all generations there was a considerable number of grade 3 chicks. During the last two years' matings practically no chicks as dark as grade 3 were obtained in the light strain. Thus at the time of crossing of the two strains in 1927 there was practically no overlapping of the distributions of the light and dark strains.

TABLE 1.—*Results of selection for light and dark down-color strains—Rhode Island Red chickens*

SELECTION FOR LIGHT STRAIN (LIGHT BY LIGHT)

Year	Number of females of grade—					Number of males of grade—				
	1	2	3	4	5	1	2	3	4	5
1925	8	10	8			9	13	6		
1926	7	28	18			8	29	14		
1927	29	29	3			39	29	3		
1928	36	8				32	7			

SELECTION FOR DARK STRAIN (DARK BY DARK)

1925			9	4				9	3	
1926		1	37	25	2		1	46	17	1
1927			29	27	1		1	23	26	2
1928			11	7	1			9	9	2

TABLE 2.—*Results of crossing of light and dark down-color strains—Rhode Island Red chickens*

F_1 GENERATION

No.	Mating	Number of females of grade—					Number of males of grade—				
		1	2	3	4	5	1	2	3	4	5
1	Dark male by light female			33	2			3	32	5	
2	Light male by dark female		9	73	7		1	12	52	8	
	Total		9	106	9		1	15	84	13	

F_2 GENERATION

3	Male from mating 1 by female from mating 1	2	12	24	10		7	3	15	5	
4	Male from mating 1 by female from mating 2	6	34	59	13	3	3	28	67	10	2
	Total	8	46	83	23	3	10	31	82	15	2
	Grand total, both sexes	18	77	165	38	5					

Reciprocal matings between the dark and light strains were made in 1927. The results of these crosses are shown in Table 2. Most of the F_1 offspring fall in grade 3, being intermediate between the two strains, but a few grade 2 and grade 4 chicks were also obtained.

The fact that the female chicks from the cross of light male by dark female are slightly lighter than those from its reciprocal might be taken to indicate that sex-linked factors are involved. However, the male offspring of this cross are also lighter, and this would not be the expected result from sex-linked inheritance. It would therefore appear that the difference between the reciprocals is more likely to be due to individual differences in the birds used as parents.

Table 2 also shows the results obtained for the F_2 generation produced in 1928. There are two series of F_2's, one resulting from the mating of F_1 females of the cross light male × dark females, and the other of females from the reciprocal cross. Both kinds of F_1 females were mated to the same F_1 male which was from the cross of a dark male by a light female. The F_1 individuals used to produce the F_2 generation were all of grade 3, this being the predominating color of that generation. The results for the F_2 generation show a distribution including the entire scale of grades. There was no significant difference between the down-color distribution of the offspring of the two lots of F_1 females. In Figure 3 are shown graphically the totals from Table 2 and the 1927 results from Table 1. The intermediate position of the F_1 graph would suggest a multiple-factor situation. However, the rapidity with which the dark and light strains were established by selection would indicate that the number of determining factors is not large. If we accept the variability occurring in the F_1 generation as an expression of the action of a single pair of genes and construct an estimated F_2 graph, we obtain one which approximates the F_2 results. The estimated graph was constructed by dividing the 303 F_2 individuals into three lots—one-fourth homozygous lights, one-fourth homozygous darks, and one-half heterozygotes. The estimated 76 lights (one-fourth of 303) were distributed over the range of color grades, shown by the 1927 light mating. In a like manner the same number of darks was distributed over the grades according to the range of the dark mating for the same year. The 151 heterozygous chicks were given a distribution modeled after the F_1 results of 1927. The estimated distributions (increasing in darkness of red from left to right) were as follows:

Grades	1	2	3	4	5
Lights	43	31	2	0	0
Darks	0	0	37	37	2
Heterozygotes	0	15	121	15	0

If the distributions for these classes are summed up, we find that the total estimated distribution corresponds relatively closely with the actual results:

Grades	1	2	3	4	5
Observed	18	77	165	38	5
Estimated	43	46	160	52	2

Although the estimated appears to fit fairly closely the observed distribution, the application of the χ^2 test [3] for goodness of fit indicates that the difference here is significant and that a single pair of genetic factors does not fully account for the results. The χ^2 value was found to be 43.86, and the value of P, 0.000000. If the above results are examined it will be seen that the greatest differences in distribution come in the first two classes. If grades 1 and 2, and 3

[3] YULE, G. N. AN INTRODUCTION TO THE THEORY OF STATISTICS. 5th ed. London.

and 4 are totaled in the estimated and obtained distributions, the fit is much better and shifts between these grades could easily have been due to errors in judgment. The χ^2 test is probably as reliable a test as can be applied to data of this kind, but in cases of extreme shifts between closely associated classes its degree of reliability can probably be questioned. In view of the above results, any attempt to estimate definitely the number of pairs of genetic factors involved in determining down color is probably unjustifiable. However, it appears to the writer that a single pair of autosomal factors will account for the major variations found in Rhode Island Red chick down color. Other minor factors probably operate, but they do not prevent the one pair of factors from accounting fairly satisfactorily

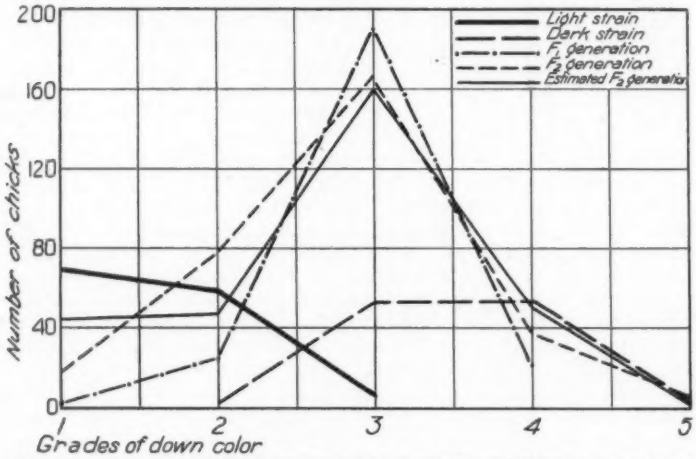

FIGURE 3.—Curves showing graphically the results of crossing light and dark color strains in Rhode Island Red chickens, drawn from figures given in Tables 1 and 2

for the results obtained. The F_1 generation shows neither condition to be dominant, since the distribution in this generation is intermediate between that of the two strains crossed.

COLOR STANDARD AND COLOR VARIATIONS

The ideal color for the Rhode Island Red breed as described in the American Standard of Perfection [4] (1923 edition) is as follows:

> The plumage * * * should be rich red, except where black is specified, though the neck, wing bows, back, and saddle of the male should be a very lustrous, rich, brilliant red in order to comply with the Standard requirements of these sections. The less contrast between hackle, wing bows, back, breast, and body, the better, as an even shade of color throughout and an harmonious blending in all sections is desired. The shade of color should neither be so light a red as to suggest orange, nor so dark as to have a brownish or violet hue. An even, rich, brilliant red is preferred.

[4] AMERICAN POULTRY ASSOCIATION. THE AMERICAN STANDARD OF PERFECTION ILLUSTRATED. A COMPLETE DESCRIPTION OF ALL RECOGNIZED VARIETIES OF FOWLS AS REVISED BY THE AMERICAN POULTRY ASSOCIATION AT ITS FORTY-SEVENTH ANNUAL MEETING AT KNOXVILLE, TENN., NINETEEN HUNDRED TWENTY-TWO. 1923 ed. 427 p., illus. n. p. 1923.

The primary wing feathers should be red like the rest of the plumage except the lower web which should be black with a red edging; the secondary wing feathers should have the black only in the upper web.

One of the common defects in the Rhode Island Red breed is that the general color is too light or too dark and the latter condition is usually accompanied by some black pigment on the surface. The breast is frequently much lighter than the rest of the plumage, this being a more common trait of females. The under color (the fluff portion of the feather which is hidden by overlapping) should match as closely as possible the general surface color, but this also varies considerably in shade, usually being lighter than the surface. The occurrence of black pigment in the under color, usually termed "smut," is a common defect. The appearance of white in the under color is also frequent, especially in males. The amount of black in wing feathers is frequently reduced or even may be entirely lacking.

The color of the eye varies from a dull gray to a reddish bay, the latter being the desired color. It was with these more common defects which were readily classifiable that the association of variations in down color was measured.

RELATION OF DOWN COLOR TO ADULT COLOR VARIATIONS

DOWN COLOR AND ADULT SURFACE COLOR

By surface color is meant the color of the back region, since the hackle and breast sections were classified separately. This is the section from which one would ordinarily get an impression of a bird's general color. The grades of surface color given in Tables 3 and 4 are those determined by comparison with the plumage standard already described. In this summary, as in all succeeding tables, the results of not only the down-color matings are given, but those of all Rhode Island Red matings for the period covered.

Since the males averaged somewhat darker than females with respect to surface color, it was necessary to tabulate the sexes separately. The results here confirm the statement made earlier that no sexual dimorphism exists with respect to down color, as the total distributions of the two sexes are very similar. In Tables 3 and 4 the darkness of the red in both down and adult plumage increases directly with the value of the grades. The coefficient of correlation [5] of down and surface color of males (Table 3), was found to be 0.179 ± 0.020. A value of so small a magnitude has, of course, no significance. In Table 4 are the data for the relationship in females of down and surface color. The coefficient of correlation was found to be 0.229 ± 0.016, and, although higher than that of males, it is too small to be considered significant. Tables 3 and 4 show that the chicks of down-color grades 1 and 2 (both of which are very light) fall in all grades of plumage color. In other words, chicks of the lightest grades of down color may as adults be some of the darkest as well as some of the lightest-colored birds of the flock. However, the two darkest grades of down color (grades 4 and 5) produced adults all but two of which were grade 3 or darker. So, although light chicks may produce very dark adults, dark chicks seldom produce light adults. The intermediate down grade 3, like the lighter grades, produced adults having almost the full range of plumage shades.

[5] BABCOCK, E. B., and CLAUSEN, R. E. GENETICS IN RELATION TO AGRICULTURE. 2d ed. New York.

TABLE 3.—*Correlation between chick down color and adult plumage color (males only)*

Grades of adult color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1		1				1
2	2	2	1			5
3	9	23	56	6	1	95
4	22	56	233	43	1	355
5	3	12	34	11		60
Total	36	94	324	60	2	

Coefficient of correlation 0.179±0.020.

TABLE 4.—*Correlation between chick down color and adult plumage color (females only)*

Grades of adult color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	1	3	4			8
2	10	25	60	2		97
3	9	59	214	35		317
4	5	25	117	27	1	175
5		1	1			2
Total	25	113	396	64	1	

Coefficient of correlation 0.229±0.016.

DOWN COLOR AND BREAST COLOR

The plumage of the breast frequently is much lighter in color than the rest of the body. This is particularly true of females, and for that reason only females were considered in calculating the relation of down color to adult breast color. From Table 5 it is seen that no significant correlation exists, the coefficient being 0.238 ± 0.015. The value of the coefficient is very similar to that obtained for females when the surface color other than that of the breast was considered.

TABLE 5.—*Correlation between chick down color and adult breast color (females only)*

Grades of adult breast color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	9	18	29			56
2	4	37	132	14		187
3	8	42	150	27	1	228
4	4	16	85	23	1	129
5		1	1			2
Total	25	114	397	64	2	

Coefficient of correlation 0.238±0.015.

DOWN COLOR AND EXCESS BLACK IN ADULT-PLUMAGE SURFACE COLOR

Particularly in strains of Rhode Island Reds which have been selected for a very dark shade of red there is a tendency to show varying amounts of black pigment on the surface. The appearance of black pigment anywhere except in the hackle of females and in wings and tail of either sex is undesirable. The males show black to a much lesser degree and for that reason only the data for females have been included in Table 6, where the relation of down-color variations to black in the plumage surface of adults is considered. This table shows the down-color distribution of those individuals showing black on the plumage surface in comparison with those free from it. The application of the χ^2 test indicates that the difference between these two distributions is not significant, since the value of P is slightly less than 0.029. In the application of the χ^2 test the method developed by Pearson [6] for comparing two independent distributions of frequency from the same population but differing in N value, was used. This method was also used for calculating the χ^2 value from Tables 9 and 10. Since there is no significant difference between the down-color distributions for birds showing and lacking excess black on the surface, it may be said that the grade of down color does not affect the occurrence of black in this region.

TABLE 6.—*Relation of down color to the occurrence of excess black in adult surface color (females only)*

Item	Number of chicks with down color of grade—				
	1	2	3	4	5
Adults showing excess black	4	30	103	30	1
Adults lacking excess black	24	84	306	48	6
	$\chi^2=8.77$		$P=0.029$		

DOWN COLOR AND UNDER COLOR

The term "under color" as used by the poultry fancier refers to the color of the fluff sections of the feather. This is the basal portion of the feather which is usually hidden by overlapping. The under color was classified into four grades and its relation to chick down color is shown in Tables 7 and 8. The fluff sections of the feathers shown in Plate 2 present the four grades of under color, 1 to 4. Table 7 gives the data for males and Table 8 that for females. For each sex there is a significant positive correlation. For males the coefficient of correlation was found to be 0.478 ± 0.008 and for females 0.517 ± 0.006. In each case these values may be considered significant, indicating that the lighter colored chicks have a tendency to develop into adults with under color which is lighter than the average. These results are clearly shown in Tables 7 and 8, for the chicks with down-color grades 1 and 2 mostly fell in adult grades 1 and 2 while the two darkest down-color grades seldom had adult plumage as light as grade 2.

[6] PEARSON, K. ON THE PROBABILITY THAT TWO INDEPENDENT DISTRIBUTIONS OF FREQUENCY ARE REALLY SAMPLES FROM THE SAME POPULATION. Biometrika 8: 250-254. 1911.

This association could not have been the result of any bias of judgment on the part of the investigator, for he recorded the adult plumage grades without knowing what the down-color grade had been.

TABLE 7.—*Correlation between chick down color and adult under color (males only)*

Grades of adult under color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	5	4	----	----	----	9
2	24	49	90	4	----	167
3	6	37	214	53	2	312
4	----	6	20	4	----	30
Total	35	96	324	61	2	----

Coefficient of correlation 0.478±0.008.

TABLE 8.—*Correlation between chick down color and adult under color (females only)*

Grades of adult under color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	4	9	1	----	----	14
2	18	72	147	8	----	245
3	3	30	218	42	1	294
4	----	3	30	15	1	49
Total	25	114	396	65	2	----

Coefficient of correlation 0.517±0.006.

DOWN COLOR AND SMUT IN UNDER COLOR

The occurrence of smut in under color is a common defect in Rhode Island Reds. In Table 9 is shown the relation of smut to down color. For determining whether the grade of down color in any way affects the occurrence of smut, the birds were divided into two classes, those showing smut and those lacking smut. If down color influences the occurrence of smut, the two classes should show a difference in distribution for down color. Table 9 shows that the individuals exhibiting smut had lighter average down color than those lacking it. This was true for both sexes and the difference between the down-color distribution for the two lots was found to be significant from the application of the χ^2 test. Although many light chicks were free from smut and there were several of the darker grades which showed it, a difference in average down color of the two lots is evident.

TABLE 9.—*Relation of down color to the occurrence of smut in adult under color*

Item	Number of chicks showing down color of grade indicated—									
	Females					Males				
	1	2	3	4	5	1	2	3	4	5
Adults showing smut	18	39	64	7	1	15	15	24	8	----
Adults lacking smut	14	79	351	69	2	22	74	305	51	2
	χ^2=50.08 P=0.000000					χ^2=37.73 P=0.000000				

DOWN COLOR AND WHITE IN UNDER COLOR

A common defect in Rhode Island Reds is the occurrence of white sections in the under color. This defect is found much more frequently in males than in females. The saddle is the section of the plumage where white more usually appears. The extent of white varies from a few feathers to large sections of the under color. Since the stock used for these studies showed white in under color somewhat infrequently in females, data are given for males only. (Table 10.) The comparison made was of individuals lacking and those having white in under color. The results show that the birds having white in under color had, as chicks, down which averaged considerably lighter than that of the birds that were free from this defect. To state the results in another way, the light-down chicks are more likely to show white in their under color as adults than the dark-down chicks. Of the 63 chicks having down of the color grades 4 and 5, only 9 showed white. Of the 127 chicks having down of color grades 1 and 2, 54 showed white and 73 showed none. The application of the χ^2 test for goodness of fit indicates that the difference obtained here is significant.

TABLE 10.—*Relation of down color to the occurrence of white in under color (males only)*

Item	Number of chicks showing down color of grade—				
	1	2	3	4	5
Adults showing white	18	36	56	9	------
Adults lacking white	17	56	269	52	2
	$\chi^2=47.7$		$P=0.000000$		

DOWN COLOR AND BLACK IN WING

The amount of black in the primary and secondary wing feathers varies widely. In some cases black is entirely absent and in others it extends over practically the whole feather. These studies have shown that there is a very close correlation between the amount of black in the primary and in the secondary feathers. As has already been stated, the classification of grade of black in wing was determined by comparison with an arbitrarily graded standard, which is shown in Figure 1. To determine whether variations in shade of down color showed any relation to the amount of black in the wing, Tables 11 and 12 were made. Since the males show considerably more black in the wing than females, the records for the two sexes are presented separately. The data in these tables are for the primary wing feathers. The results on secondaries are not given since they are very similar. In neither case is the correlation significant, although in Table 12 there appears to be a slight tendency among the males for those in the darker wing grades to have darker down. The coefficient of correlation for males was 0.162 ± 0.028 and for females 0.133 ± 0.020.

TABLE 11.—*Correlation between chick down color and amount of black in adult wing feathers (females only)*

Grades of black in adult wing	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	.....	18	34	8	.....	69
2	25	52	210	29	1	310
3	4	37	125	21	2	184
4	2	5	31	12	.....	52
5	.....	4	13	7	.....	20
6	.....	1	1	.....	.....	7
Total	31	117	414	77	3	.....

Coefficient of correlation 0.133±0.020.

TABLE 12.—*Correlation between chick down color and amount of black in adult wing feathers (males only)*

Grades of black in adult wing	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	1	1	13	3	.....	18
2	19	37	92	11	2	161
3	9	23	79	7	.....	118
4	4	15	79	24	.....	122
5	2	18	58	14	.....	92
6	1	.....	2	1	.....	4
Total	36	94	323	60	2	.....

Coefficient of correlation 0.162±0.028.

DOWN COLOR AND EYE COLOR

The eye color most sought for by the poultry fancier is a brilliant reddish bay. There are found, however, varying shades of red in the eye of Rhode Island Reds. In some cases the red color may be entirely lacking, leaving the eye a light gray. The color referred to is that of the iris. In addition to variations in the amount of red there are differences in distribution of color in the different grades of eye color. The reduction of the red pigment is first seen at the margin of the pupil and the last traces are usually found at the outer edge of the iris. As was true of the plumage, the eye color was not graded until the 6-months age was reached, after which there appears to be very little change. Plate 2 shows from right to left the order of disappearance of red from the eye. Table 13 presents the relationship of down color to eye color. The two sexes showed the following distribution for eye color:

Males	5	55	177	183	93	5
Females	10	87	244	208	89	4

TABLE 13.—*Correlation between chick down color and adult eye color (both sexes)*

Grades of adult eye color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	.....	2	6	7	.....	15
2	.....	19	101	21	1	142
3	21	80	270	50	.....	421
4	28	80	242	38	3	391
5	19	28	113	21	1	182
6	.....	1	7	1	.....	9
Total	68	210	739	138	5	.....

Coefficient of correlation −0.123±0.015.

The grades become progressively darker from left to right. The results show no relationship between adult eye color and chick down color, since the coefficient of correlation -0.123 ± 0.015 is statistically insignificant.

DOWN STRIPING AND ADULT PLUMAGE COLOR

Although Rhode Island Red chicks are usually of a more or less uniform red color above, stripes occasionally occur on the back. These are ordinarily brownish in color and vary from definite stripes to small irregular spots. Chick A in Plate 1 shows striping. Striping occurs rather infrequently in the stock studies, so data are available on only a limited number of individuals. The records showed the striped chicks to be preponderantly females (29 females to 5 males). Although the sex ratio is distinctly aberrant, the difference may be a chance one resulting from the small numbers. The records for the 34 chicks that showed striping were studied in relation to the quality of the adult plumage. Much of the data on striping were taken before definite standards were used, and therefore did not lend themselves to uniform tabulation. However, an examination of the descriptions showed that the striped chicks did not differ from the average of other chicks with respect to adult surface color since the range was from light to dark individuals. The distributions for black in wing and eye color were also practically normal. The under color of the striped chicks was, however, found to be of very poor quality. The records for these chicks show that 6 had good red under color, 10 medium, and 18 poor. The quality here is far below the average for the nonstriped chicks. The explanation of this poor quality is found in the data on the occurrence of smut and white in the under surface. Twenty were listed as showing much smut, 8 some smut, 1 slight smut, and 5 no smut. Four showed much white, 10 some white, 5 slight white, and 15 no white. In all, 29 of the 34 showed some smut and 19 showed some white in the under surface. Considering all of the descriptions taken, less than 15 per cent of the females showed smut in the under surface, and the males showed much less. Of the striped female chicks over 85 per cent carried smut. Since so few females showed white in under surface no summaries were made for this sex in the study of white in under color. Although most of the striped chicks were females, over half of them showed some white in under color. One fact which should be taken into consideration is that striping is much more conspicuous in the lighter colored chicks and most of those listed as striped were of the lighter grades. Since it has already been shown that there is a relationship between down color and under color and also between down color and smut, the grade of down may also be a factor here. It can be said, however, that most of the striped chicks had poor quality of under color.

SUMMARY AND CONCLUSIONS

This study was conducted to determine whether Rhode Island Red chick down-color variations were heritable, and, if so, how they were inherited. It was also the purpose of the study to learn whether the variations found in down color bore any relation to adult plumage color variations. Any relationship found would, of course, be of

value in predicting, at hatching, the quality of adult plumage color to be expected. The relationship found between down and adult plumage color are purely physiological and not of the nature of genetic linkages.

It was found that variations in the shade of the down color are inherited and that it was possible to establish strains which bred relatively true for the dark and light red down. A single genetic-factor difference seemed sufficient to explain most variations encountered when the light and dark strains were crossed, neither condition being dominant.

The relationship of down-color variations to the following variations in adult plumage color were determined—general surface color, occurrence of black on the surface, breast color, under color, smut in under color, white in under color, black in the larger wing feathers, and eye color. The only significant correlation was found between the shade of down color and under color, and from this it was learned that a high percentage of the light-colored chicks developd into adults with light under color. In the same way most of the adults with the darker shades of under color showed dark-red down color as chicks. The lightest colored chicks also showed a tendency to develop into adults with under color showing much smut and white. Chicks showing conspicuous striping at hatching are likely to develop into adults with under color of inferior quality, usually carrying considerable smut and white in that section. No agreement was found between the shade of the adult surface color and down color. The chicks having the lightest shades of down might develop into very dark adults. It was found, however, that the dark chicks seldom produced adults of the lighter shades of red.

From a practical point of view, then, the light-colored Rhode Island Red chicks should be eliminated, primarily because of their effect upon the under color of the flock. If only individuals showing the darker shades of down are reared an improvement in under color may be expected. In order to eliminate smut and white from the under color, chicks showing striping should not be reared. If only chicks of the very darkest shades of down color are retained, the lighter colored adults will be avoided.

VARIETAL RESISTANCE OF SOYBEAN TO THE BACTERIAL PUSTULE DISEASE [1]

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INTRODUCTION

The soybean, *Soja max* (L.) Piper, is susceptible to parasitic attack by a number of plant pathogenes. One of these, *Bacterium phaseoli sojense* Hedges, the causal agent of the bacterial pustule disease,[2] is widespread and occurs in a majority of the soybean fields in North Carolina. The organism overwinters in diseased leaf material [3] and on seed from diseased plants.[4] Seed treatments have served to delay the time when the disease became most abundant in a given season, but in no case has seed treatment given complete or even satisfactory control. The disposal of dead, diseased leaf material by plowing after harvest and the rotation of crops as generally practiced are undoubtedly valuable aids to control, but the disease has on a number of occasions developed in soybean test plots which had been planted with treated seed after an interval of at least one season in which no soybeans were grown on the land. In view of this situation it seemed desirable to determine if any of the various varieties or strains under cultivation showed more than usual resistance to the bacterial pustule disease. Accordingly, field observations were made and certain experiments were conducted in order to gather a body of information bearing definitely on this point. The data collected from these observations and experiments are presented in the following pages.

FIELD OBSERVATIONS

Observations were made at random in the field in the summers of 1925, 1926, and 1927 wherever a number of varieties of soybean were found growing together. The presence or absence of bacterial pustule was recorded and usually the relative amount of disease on the different varieties was estimated. The result of the observations made in the late summer of 1927 on plants growing in three variety tests are given in Table 1 as an example of the findings obtained in each of the three seasons.

This table shows that most of the varieties grown in these tests are susceptible to bacterial pustule. Such varieties as Dixie, Easycook, Ebony, Hahto, Hollybrook, Morse, and Peking showed the greatest amount of disease. Still others, as Austin, Virginia, Goshen, Haberlandt, Herman, Merko, and Pine Dell Prolific, were somewhat

[1] Received for publication Apr. 2, 1929; issued November, 1929. Published with the approval of the director of research as paper No. 33 of the Journal Series.

[2] HEDGES, F. BACTERIAL PUSTULE OF SOYBEAN. Science (n. s.) 56: 111-112. 1922.
HEDGES, F. A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI EFS. Jour. Agr. Research 29: 229-251, illus. 1924.
WOLF, F. A. BACTERIAL PUSTULE OF SOYBEAN. Jour. Agr. Research 29: 57-68, illus. 1924.

[3] LEHMAN, S. G. STUDIES ON THE BACTERIAL PUSTULE DISEASE OF SOYBEAN. (Unpublished manuscript.)

[4] WINTERS, R. Y. FIFTIETH ANNUAL REPORT OF THE NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION FOR THE FISCAL YEAR ENDED JUNE 30, 1928. STATISTICAL SUMMARY FOR YEAR ENDING DECEMBER 1, 1928. N. C. Agr. Expt. Sta. Ann. Rpt. 51: 60. 1929.

less severely infected but still showed considerable injury from the disease. Two varieties, Lexington and Columbia, were entirely free from bacterial pustule in the one test from which records for these varieties were secured.

TABLE 1.—*Relative amount of bacterial pustule disease observed on different varieties of soybean growing in the field in three localities of North Carolina, in 1927*

Variety	Relative amount of disease at—			Variety	Relative amount of disease at—		
	Willard	Raleigh	Moyock		Willard	Raleigh	Moyock
A. K.	(a)	Little		Laredo	Much	Much	
Austin		Much	Much.	Lexington		None	
Biloxi	Little	Little		Mammoth Yellow	Little		Little.
Chiquita	do	do	Do.	Mammoth Brown	Much	Little	
Columbia		None		Merko		Much	
Dixie		Very much.		Morse		Very much.	
Easycook	Very much.			Otootan	Little	Much	Do
Ebony		Very much.		Old Dominion	do	Little	
George Washington		Much		Peking		Very much.	
Goshen		do		Pine Dell Prolific		Much	
Goshen Prolific		do		Sooty	Little	do	
Government		do		Southern Prolific	Much	Little	
Haberlandt				Tarheel Black	do	do	Much.
Hahto	Very much.		Do.	Tokyo	do	do	Little.
Herman	Much	Much		Viriginia	do	Much	Do.
Hollybrook		Very much.		Wilson		Little	Do.
				Yokoten		Much	

[a] Varieties for which no data are recorded at a given place either had not been included in the test at that place or had reached maturity and shed their leaves at the time the observation was made.

Data such as those shown in Table 1, obtained by more or less random observation, are not very satisfactory as a basis for judging the disease resistance of different varieties of a given crop. For example, Mammoth Brown at Willard showed "much" disease while at Raleigh it showed "little." On the other hand, Otootan showed "little" at Willard and "much" at Raleigh. The reason for these differences in the amount of disease on the same variety growing in different locations may be conjectured to be due to any one of several causes, as, for example, to differences in amount of natural inoculation, or to variations in susceptibility of different strains of the host plant, or to differences in degree of maturity of the host at the time the organism causing the disease was being disseminated. In order to obviate these differences to as great an extent as possible it seemed desirable that further data should be obtained from tests in which the varieties used were artificially inoculated with an abundance of the causal organism. By this method data could also be secured on a larger number of varieties. Two such tests, one in the greenhouse and one in the field, were made.

GREENHOUSE VARIETY TEST

In the greenhouse test 10 seeds of each variety were planted at a marked location in sandy soil in the greenhouse bench. After the plants had attained suitable size they were inoculated on several occasions by spraying with a water suspension of young cultures of

Bacterium phaseoli sojense previously isolated from a typical pustulate lesion taken from a leaf of the Biloxi variety. Just previous to each inoculation the soil of the bench was thoroughly wetted, and immediately following inoculation the bench was covered with a muslin cloth kept moist by repeated sprinkling over a period of three to four days, at the end of which time the cloth was removed. The seeds were planted on December 20, 1927, and the first inoculation was made on January 24, 1928. Subsequent inoculations were made on January 31, February 13, March 14, March 16, and March 20. The varieties of soy beans used in the test and the results obtained are recorded in Table 2.

TABLE 2.—*Relative resistance of different soybean varieties to infection by Bacterium phaseoli sojense in a greenhouse variety test, 1928*

Variety	Ratio of number of plants infected to number inoculated, intensity of disease and coefficient of susceptibility, on dates indicated							Group No.
	Feb. 21		Mar. 30		Apr. 11			
	Ratio	Intensity	Ratio	Intensity	Ratio	Intensity	Coefficient	
Austin	1:8	1	------	(a)	------	------	------	------
A. K.	3:9	------	3:9	a b 1	7:9	1	2.05	2
Black Eyebrow	0:6	------	c 4:5	b 1	5:5	2	5.00	3
Biloxi	4:9	2	c 7:8	b 1	8:8	2	5.00	3
Chestnut	0:6	------	0:6	(d)	4:6	1	1.65	2
Columbia	0:1	------	0:1	(b)	0:1	------	.00	1
Chiquita	0:7	------	3:7	e 2	7:7	2	5.00	3
Dixie	0:6	------	c 1:5	b 1	4:5	2	4.00	2
Ebony	0:7	------	0:7	(d)	3:7	1	1.07	2
Goshen Prolific	2:10	2	------	(a)	9:9	5	12.50	4
George Washington	0:7	------	0:7	(b)	6:6	1	2.50	2
Government	0:4	------	0:4	(c)	0:4	------	.00	1
Haberlandt	5:10	5	10:10	e 8	10:10	8	20.00	4
Hamshet f	0:4	------	1:4	c 1	3:4	1	1.87	2
Hollybrook	7:8	6	8:8	b 5	8:8	5	12.50	4
Habaro	1:7	1	c 2:6	d 1	6:6	1	2.50	2
Johnson	4:5	------	c 4:4	c 1	4:4	1	2.50	2
Lexington	3:8	1	3:3	b 1	3:3	2	5.00	3
Laredo	0:7	------	0:7	(b)	5:8	1	1.55	2
Morse	4:9	1	4:8	b 1	9:9	2	5.00	3
Merko	2:10	3	2:10	(a)	7:10	1	1.75	2
Mammoth Yellow	0:7	------	6:7	b 2	7:7	2	5.00	3
Manchu	0:7	------	0:7	(c)	5:7	1	1.77	2
Mammoth Brown	0:7	------	c 4:5	c 1	5:5	3	7.50	3
Old Dominion	0:6	------	0:6	(c)	0:6	------	.00	1
Otootan	0:9	------	6:9	b 1	8:9	2	4.40	2
Peking	0:8	------	0:8	a (b)	0:8	------	.00	1
Pine Dell Prolific	1:7	1	1:7	b 1	6:6	2	5.00	3
Southern Prolific	0:9	------	0:9	(c)	8:9	1	2.20	2
Tarheel Black	3:10	3	7:10	b 5	8:10	5	10.00	4
Tarheel Black	8:9	3	8:9	b 1	9:9	2	5.00	3
Tokyo	0:7	------	3:7	b 1	6:7	2	4.25	2
Virginia	10:10	5	------	(a)	------	------	12.50	4
Wilson	7:9	------	------	(a)	------	------	------	------
Wilson Black	1:8	1	2:8	(a)	------	------	------	------
Yokoten	0:6	------	6:6	b 3	6:6	3	7.50	3
54610	1:7	1	1:7	(b)	7:7	1	2.50	2
37261	0:9	------	c 6:7	b 2	7:7	2	5.00	3
36906-A-7-10	0:6	------	6:6	b 3	6:6	3	7.50	3
36906-A-7-5-1	0:8	------	1:8	b 1	6:8	1	1.87	2

a Top leaves badly injured from becoming too dry.
b Growing rapidly, foliage tender.
c Remainder of plants died since last record was taken.
d Stunted, not growing properly, apparently because of unfavorable length of day.
e Growing but less rapidly than varieties marked *b*, and possessing less succulent leaves.
f Authors uncertain of correctness of this name.

In this table infection records are presented in two columns under three different dates. The fractions in the first column are the ratios of diseased to inoculated plants, the denominator being the number of plants inoculated, the numerator the number which became infected on one or more leaves. The numbers in the second column under each date are intended to convey an idea of the intensity of the disease as indicated by the estimated relative number of lesions per leaf, the larger integers indicating proportionally larger numbers of lesions. The coefficients of susceptibility shown in the third column under date of April 11 were calculated for each variety by the following formula:

$$\frac{\text{Number of infected plants (or leaves)}}{\text{Number of inoculated plants (or leaves)}} \times \text{intensity} \times 2.5$$

By this formula the two factors used to measure susceptibility are combined in a single multiple for the purpose of facilitating comparison between varieties. The constant 2.5 is used only in Table 2. The range of the values used to indicate intensity of infection in Table 3, which is to be compared with Table 2, is 2.5 times as great in the former as in the latter; hence the true coefficients in the former are 2.5 times as great as in the latter for the same varieties. Introducing this factor 2.5 into the formula used for Table 2 and omitting it for Table 3 removes this difference and greatly facilitates comparison of the results recorded in the two tables. The different varieties are ranked and assigned a group number, using the coefficients of susceptibility as a basis of comparison. The varieties having the lowest coefficients of susceptibility are placed in Group 1; those with highest coefficients, in Group 4. The different groups thus arbitrarily established are shown in Table 4.

During the interval between the first inoculations in the greenhouse variety test and the time of taking the first data on February 21, the temperature of the greenhouse was too low for a high percentage of infection to occur. However, a comparatively small number of infections were found on the most susceptible varieties. By March 30, three additional inoculations having been made in the meantime, a considerable increase in the number of diseased plants had occurred. The proportion of diseased to healthy plants was still low, however. Because there was so little disease up to March 30, the data recorded on that and the previous date are not used in determining coefficients of resistance, but are presented to show the slow increase of disease with ameliorating temperature conditions and to corroborate the findings based on the data taken April 11. For several days previous to April 11, when the last records were taken, the daytime temperatures had been uniformly high and the nights warm, resulting in development of the disease on nearly all the plants. The grouping of the varieties in relation to resistance is based on the data taken on this date.

TABLE 3.—*Relative resistance of different soybean varieties to infection by Bacterium phaseoli sojense in field experiment, 1928*

Variety	Ratio of number of plants or leaves infected to number inoculated, intensity of disease, and coefficient of susceptibility on dates indicated						Average coefficient	Group No.
	August 1			September 24				
	Ratio (plants)	Intensity	Coefficient	Ratio (leaves)	Intensity	Coefficient		
Austin	38:38	8	8.00	[a]			8.0	3
A. K	54:58	4	3.72	1.0	10	10.0	6.8	3
Aksarben	21:23	4	3.64	1.0	10	10.0	6.8	3
Black Eyebrow	54:54	2	2.00	1.0	8	8.0	5.0	3
Biloxi	59:59	8	8.00	.75	5	3.75	5.8	3
Barchet	11:15	4	2.92	.8	5	4.0	3.4	2
Chiquita	30:30	5	5.00	.9	5	4.5	4.7	2
Chestnut	50:50	10	10.00	1.0	10	10.0	10.0	4
Columbia	2:22	1	.09	Trace			0.9	1
Dixie	18:19	5	5.00−	1.0	15	15.0	10.0	4
Ebony	45:45	15	15.00−	1.0	12	12.0	13.5	4
Easycook	13:13	1	1.00−	.5	2	1.0	1.0	2
George Washington	22:22	8	8.00−	1.0	15	15.0	11.5	4
Goshen Prolific	77:77	20	20.00−	.8	10	8.0	14.0	3
Government	43:44	5	5.00−	.8	5	4.0	4.5	2
Herman	16:16	8	8.00	1.0	18	18.0	13.0	4
Hamshet [b]	4:4	8	8.00	[a]			8.0	3
Haberlandt No. 12	63:63	5	5.00	1.0	10	10.0	7.5	3
Hollybrook	55:55	10	10.00	1.0	18	18.0	14.0	4
Habaro	28:28	5	5.00	1.0	10	10.0	7.5	3
Hoosier	30:30	10	10.00	1.0	15	15.0	12.5	4
Hamilton	4:4	4	4.00	1.0	15	15.0	9.5	3
Hahto	14:14	2	2.00	.8	5	4.5	3.2	2
Ito San	11:11	2	2.00	[a]			2.0	2
Johnson	25:25	8	8.00	10.0	10	10.0	9.0	3
Laredo	30:30	5	5.00	.6	2	1.2	3.1	2
Lexington	37:37	5	5.00	1.0	12	12.0	8.5	3
Mammoth Brown	42:42	8	8.00	1.0	10	10.0	9.0	3
Morse	71:72	5	−5.00	1.0	8	8.0	6.5	3
Manchu	32:32	5	−5.00	1.0	8	8.0	6.5	3
Merko	42:42	10	10.00	1.0	20	20.0	15.0	4
Mammoth Yellow	56:56	5	5.00	1.0	5	5.0	5.0	3
Midwest	27:30	8	7.20	1.0	15	15.0	11.1	4
Mandarin	7:8	1	.87	[a]			.8	1
Medium Green	20:20	4	4.00	1.0	15	15.0	9.5	3
Mikado	22:23	2	−2.00	1.0	10	10.0	6.0	3
Minsoy	5:5	2	2.00	1.0	20	20.0	11.0	4
Otootan	18:18	10	10.00	1.0	10	10.0	10.0	4
Old Dominion	19:19	3	3.00	1.0	5	5.0	4.0	2
Pine Dell Prolific	46:46	8	8.00	1.0	18	18.0	13.0	4
Peking	28:30	4	2.82	1.0	10	10.0	6.4	3
Southern Prolific	52:52	5	5.00	1.0	15	15.0	10.0	4
Tokyo	52:52	5	5.00	.9	5	4.5	4.7	2
Tarheel Black	35:47	1	.74	.5	2	1.0	.8	1
Virginia	77:77	15	15.00	1.0	12	12.0	13.5	4
Wilson	72:72	10	10.00	1.0	10	10.0	10.0	4
Wilson Black	35:36	5	−5.00	.9	5	4.5	4.7	2
Wilson Five	6:34	2	.34	.8	5	4.0	2.1	2
Wea	9:9	2	2.00	[a]			2.0	2
Yokoten	24:24	5	5.00	1.0	20	20.0	12.5	4
35906–A–7–5–1	26:26	5	5.00	1.0	10	10.0	7.5	3
35906–A–7–10	17:17	5	5.00	1.0	5	5.0	5.0	3
37261	43:43	8	8.00	1.0	15	15.0	11.5	4
54610	12:12	5	5.00	1.0	5	5.0	5.0	3
Yokoten [b]	3:3	4	4.00	.8	2	1.6	2.8	2

[a] This variety had matured and shed its leaves at this date.
[b] Authors uncertain of correctness of this name.

TABLE 4.—*Groups into which soybean varieties were classified on a basis of the coefficient range and their relative resistance to infection by Bacterium phaseoli sojense*

Group No.	Coefficient of susceptibility	Resistance
1	Less than 1	High.
2	1 to less than 5	Moderately high.
3	5 to less than 10	Moderately low.
4	10 to 20	Low.

Forty varieties and selections were included in this test. Of this number 4, Columbia, Government, Peking, and Old Dominion, are placed in Group 1 for high resistance; 16, in Group 2 for moderately high resistance; 12, in Group 3 for moderately low resistance; 5, in Group 4 for low resistance; and 3 are given no group number because of insufficient data. It is probable, as indicated in Table 1, that the variety Peking in Group 1 would have fallen into Group 2, 3, or 4 had not the top leaves been injured by severe drying about the time temperatures in the greenhouse were becoming favorable for infections to occur. The plants of the varieties Old Dominion and Government were 8 to 10 inches tall on March 30. At that time they possessed four and five trifoliate leaves, respectively, and were growing but not so rapidly as some other varieties. It is probable that under conditions more favorable for growth and for occurrence of infections these plants would have fallen in Group 2 or 3. Only one plant of the variety Columbia came up. On March 30 this plant was growing rapidly, having produced nine trifoliate leaves on a stem 24 inches long. The tender succulent character of this plant was very favorable for infection, the success of which was very probably thwarted by the factor of inherent resistance. In general, the plants falling in Groups 3 and 4 were growing well and possessed tender foliage. On the other hand, some of the varieties placed in Group 2, notably Chestnut, Ebony, Habaro, and Manchu, showed a stunted condition accompanied by very slow growth and early pod development, due supposedly to a photoperiod unfavorable for the best development of these varieties at this season of the year. Had conditions been favorable for more rapid growth, such for instance as occurs in the field in the summer months, these four varieties probably would have shifted to a place with the less resistant varieties in Group 3 or Group 4.

FIELD VARIETY TEST

The seeds used in the field experiment were planted in sandy loam soil on bottom land adjacent to a small creek. Seeds of most of the varieties were planted on June 18, 1928; the remainder, on July 2.[5] The plants were inoculated by spraying them on several occasions with suspensions of *Bacterium phaseoli sojense* of the same strain used in the greenhouse experiment described above. Late afternoon or evening and, as often as possible, rainy days were chosen as the most fitting time for applying the bacteria to the plants. The first inoculation was made in the evening of July 12, a cloudy, showery

[5] Acknowledgment is made of the kind cooperation of William J. Morse, of the U. S. Department of Agriculture, who furnished seed of 15 of the varieties used.

day. This inoculation was confined to the varieties planted on June 18, since these plants had two to three trifoliate leaves apiece, while some of the varieties planted on the later date, July 2, were just coming through the ground. The second inoculation was made on July 17, at which time all the varieties were sprayed with the bacterial suspension. Other inoculations were made on one subsequent date in July and on three in August.

As a means of testing the virulence of the bacterial suspension used for the several inoculations, a pot of plants of the Herman variety was inoculated each time and kept for a few days under a bell jar in the greenhouse. In each instance the disease developed abundantly on the check plants in the greenhouse.

On August 1, and again on September 24, the plants in the field were examined in order to determine the amount of disease present. The data obtained are recorded in Table 3. With the exception of two varieties, Columbia and Wilson Five, all or practically all of the plants of each variety in the test were diseased by August 1. By September 24 the plants of many of the varieties had become so large and tangled that counting was not feasible, and it was thought that an estimation of the percentage of diseased leaves would serve as a better basis of comparison. These data, originally taken as percentage of diseased leaves, are recorded not in terms of percentage but as the equivalent decimal fraction (column 5). This decimal expresses the ratio of diseased to inoculated leaves. The coefficient of susceptibility is calculated for each variety at each time of taking the data. The average of the two coefficients is employed to determine the rank or group number of each variety using as a basis the scale given in Table 4.

Fifty-five varieties were tested in this experiment. Of this number, 3 fall into Group 1 for high resistance; 13 into Group 2 for moderately high resistance; 22 into Group 3 for moderately low resistance; and 17 into Group 4 for low resistance. The three most resistant varieties, as indicated by this test, are Columbia, Mandarin, and Tarheel Black. On September 24, when the second set of data were taken, the variety Mandarin was mature and had shed all its leaves. It is quite possible that this variety would have fallen into Group 2 if a second set of data had been taken previous to the shedding of its foliage. In regard to the proper placing of the variety Tarheel Black, the field and the greenhouse inoculation experiments are not in agreement. In the field this variety, as noted above, fell into Group 1, while the results obtained in the greenhouse experiment placed it in Group 4. General field observations, as recorded from time to time (Table 1), indicate that Tarheel Black is not highly resistant to bacterial pustule but should be put in Group 2 or possibly Group 3 rather than in Group 1.

A summary of the results of the greenhouse and field tests is presented in Table 5. Of the 16 varieties placed in Group 2 in the greenhouse test, only 2, Laredo and Tokyo, fall into that group in the field test, the remaining 14 falling equally in Groups 3 and 4. Seven of the 13 varieties in Group 2 of the field test were not included in the greenhouse test; 2 were from Group 1, 2 from Group 2, 1 from Group 3, none from Group 4, and 1 was not classified. Of the 12 varieties in Group 3 of the greenhouse test, 2 shift to higher groups in the field tests, 7 remain in Group 3, and 3 shift to Group 4. Only 3 of the 5

varieties in Group 4 of the greenhouse test shift to higher groups in the field, while of the 17 in Group 4 in the field test, 7 came from Group 2 and 3 from Group 3 of the greenhouse test. There is evident a general shifting of varieties from groups of high resistance into groups of lower resistance with but little shifting in the opposite direction when the experiment is transferred from the greenhouse to the field. Such a shift is to be expected when one considers that the greenhouse experiment was conducted in winter and early spring when temperature and other conditions were not so favorable for infection as in the field in summer.

TABLE 5.—*Summary of results of greenhouse and field tests on the resistance of different soybean varieties to infection by Bacterium phaseoli sojense*

Group No.	Number of varieties in each resistance group		Varieties which are same in each experiment		Number of varieties in greenhouse test shifting to other groups in field test	
	Greenhouse experiment	Field experiment	Number	Name	To higher resistance group	To lower resistance group
1	4	3	1	Columbia	0	3
2	16	13	2	Laredo, Tokyo	0	14
3	12	22	7	Black Eyebrow, Biloxi, Morse, Lexington, Mammoth Yellow, Mammoth Brown, 35906–A–7–10	2	3
4	5	17	2	Hollybrook, Virginia	3	.....
Total	*40	55	12	.....	5	20

*Includes three varieties not classified.

Both the field and greenhouse tests indicate that the variety Columbia holds first place among the varieties tested for resistance to bacterial pustule. This finding is in harmony with the observation recorded in Table 1, in which Columbia was free from bacterial pustule when varieties in near-by rows showed high infestation. Of the other three varieties, Government, Old Dominion, and Peking, which fell in Group 1 in the greenhouse variety test, Government and Old Dominion fall into Group 2 and Peking in Group 3 in the field test.

The varieties Hollybrook and Virginia fall in Group 4 in both experiments. This fact would seem to mark them as the most susceptible of the varieties tested. However, notes taken in the field but not shown in Table 3 indicate that the varieties Yokoten, Minsoy, Merko, and Pine Dell Prolific were injured quite as badly as Hollybrook and perhaps more than Virginia. Lesions were very numerous on the leaves of all these varieties and a large proportion of the leaf tissue was killed by confluence of diseased areas.

GREENHOUSE TEST WITH COLUMBIA

As a further test of the resistance of the variety Columbia to the bacterial pustule disease, young plants growing in pots in the greenhouse and having one trifoliate and two unifoliate leaves open were

inoculated on January 30, 1929, by rubbing their upper and lower surfaces with a heavy suspension of the pustule organism. The plants were immediately placed under bell jars and kept there for four days in order to maintain a condition of high relative humidity favorable to infection. During the infection and incubation period the greenhouse was kept at a temperature previously determined to be favorable for development of the disease. As a check on the virulence of the cultures used, young plants of the Herman variety were inoculated by the same method and with the same bacterial suspension used on Columbia. Still another check of Herman was inoculated in the same manner but with a nonparasitic bacterium to test the effect of such mechanical injuries to the leaves as might result from handling and rubbing them in the process of inoculation. Both checks of the Herman variety were subjected to the same conditions of humidity and temperature as those of the variety Columbia.

At the end of nine days no infections could be detected on Columbia, but infections on Herman plants inoculated with *Bacterium phaseoli sojense* were very numerous, and some of the leaves were dying because of the great number of lesions. The bacteria entered quickly and produced rapid injury at places of mechanical abrasion, but large numbers of lesions resulted from stomatal infection also. Some mechanical injury was apparent on the Herman plants inoculated with the nonparasitic bacterium, but no symptoms typical of lesions of bacterial pustule developed about these injuries nor did any of the leaves die. Likewise, mechanical abrasions were apparent on the plants of the Columbia variety, but no bacterial infections occurred at any time at the points of injury on these plants, not did any of the leaves die from mechanical or pathological effects resulting from the inoculation.

By the sixteenth day, however, a comparatively small number of infections (about 50 in all) had occurred on the variety Columbia. All of these were on trifoliate leaves, the great majority occurring on two small areas of the 12 trifoliate leaflets inoculated. No infections were present on the unifoliate leaves of Columbia, while on the corresponding leaves of the Herman variety lesions were very numerous. From this it appears that the very young leaves of the Columbia variety are susceptible to infection under especially favorable conditions and that they lose this susceptibility more quickly than the leaves of Herman.

A very obvious difference was apparent in the size and appearance of the lesions on the leaves of the Columbia and Herman varieties. The lesions produced by the bacterial pustule organism are normally characterized by the development of a pustule the base of which is circumscribed by a yellow halo. The pustule usually soon ruptures or dries down and a brown or reddish spot appears. This brown spot may increase considerably in size, but the yellow halo is always an evident and often the most conspicuous feature of the lesion. On the variety Columbia, in the experiment recounted above, only the faintest suggestion of a yellow halo could be seen about some of the pustules, while about most of them no evidence of a halo could be distinguished, the spot being no wider than the base of the pustule itself. On the leaves of Herman, however, the yellow halo was strongly evident, the brown necrotic areas in the centers of the lesions were broader, and the estimated average total width of the lesions

was three to four times that of the lesions on Columbia. This practically complete suppression of the yellow halo and the smaller size of the lesions may be taken as amother indication of resistance on the part of the variety Columbia.

DISCUSSION AND CONCLUSIONS

Random field observations and more carefully executed artificial inoculation tests in the greenhouse and in the field have yielded certain data indicative of the relative resistance of 56 different varieties of soybean to the bacterial pustule disease. Lists of the varieties which possess highest, intermediate, and lowest resistance, as judged by the writer from general field observation and experimental tests, are given below. The varieties in each list are arranged in the order of decreasing resistance, the least resistant coming last.

Highest	Intermediate	Lowest
Columbia	Laredo	Hoosier
Mandarin	Chiquita	Midwest
Old Dominion	Mammoth Yellow	Medium Green
	Tarheel Black	Virginia
	Biloxi	Herman
	Otootan	Haberlandt
	Goshen Prolific	Pine Dell Prolific
	Southern Prolific	Hollybrook
		Minsoy
		Merko
		Yokoten

Minsoy, Hollybrook, Merko, and Yokoten are probably the most susceptible. Herman, Virginia, Pine Dell Prolific, and Midwest are also highly susceptible. Aside from Herman and Virginia, the varieties most commonly cultivated in North Carolina, namely, Laredo, Otootan, Mammoth Yellow, Biloxi, and Tarheel Black, are intermediate in resistance.

The variety Columbia is highly resistant to the particular strain of the bacterial pustule organism used in the tests described in this paper. This high resistance is indicated by the results of the greenhouse and the field variety tests. Although only 1 plant of this variety was available for use in the greenhouse variety test, this plant grew rapidly and was in a tender condition favorable for infection during the whole period of the test. It had produced nine leaves on a stem 24 inches long and was larger than the plants of any other variety on March 30, when the last disease records were taken. Because of its large size and numerous leaves it could not have escaped a liberal application of inoculum. That this variety is not completely resistant or immune to bacterial pustule is shown by the occurrence of lesions on 2 of the 22 plants examined in the field on August 1, and on the plants of this same variety inoculated in the second greenhouse test on January 30, 1929. In this second greenhouse test conditions very favorable for infection were maintained, with the result that some infections did occur on Columbia. However, the amount of disease on Columbia was small compared with that on Herman. The very young leaves of Columbia are susceptible under conditions very favorable for infection, but they lose this susceptibility much earlier than those of more susceptible varieties. This is indicated by the occurrence of numerous lesions on the unifoliate

leaves of Herman, while the corresponding leaves of Columbia remained free. The relatively small number of lesions which did occur on Columbia in the second greenhouse test, the slowness with which they appeared, and the nearly complete suppression of the customary yellow halo are indications of the relatively high resistance of this variety.

The same strain of *Bacterium phaseoli sojense* was used in all the tests described in this paper. This strain (S. B. P. 8 Col. A) was isolated on August 28, 1927, from a young lesion on a leaf of the Biloxi variety. It will be of interest to learn whether this variety is equally resistant to all strains of this organism.

According to Piper and Morse,[6] the variety Columbia was introduced in to America from Paotingfu, China, in 1908. It is a green-seeded variety which produces a rather stout erect growth and bears both purple and white flowers. It matures in about 125 days. Columbia is not grown on the farms of North Carolina, but it is hoped that its resistance will be found to encompass all strains of the bacterial pustule organism, and that it may be used as a parent in the work of combining the quality of high resistance to bacterial pustule with the desirable characters of the more popular varieties of soybeans now being grown.

SUMMARY

Certain observations have been made relative to the natural occurrence of the bacterial pustule disease of soybean in variety tests in the seasons of 1925, 1926, and 1927. A record of the relative amounts of disease occurring on 33 different varieties observed in 1927 is given.

Artificial inoculation tests were made in 1928 in which 40 varieties of soybeans growing in the greenhouse and 55 varieties planted in the field were sprayed on several occasions with heavy suspensions of *Bacterium phaseoli sojense*. Columbia was found to possess greater resistance to infection by this bacterium than any other variety used in these tests.

Infections did occur on Columbia when inoculated plants were kept under especially favorable conditions, but the lesions developed more slowly, were smaller and fewer in number than on resistant varieties, and development of the halo was almost completely suppressed.

The varieties Hoosier, Midwest, Medium Green, Virginia, Herman, Haberlandt, Pine Dell Prolific, Hollybrook, Minsoy, Merko, and Yokoten are the least resistant of the varieties tested. The more commonly cultivated varieties Laredo, Chiquita, Mammoth Yellow, Tarheel Black, Biloxi, Otootan, Gosehn Prolific, and Southern Prolific are intermediate in resistance.

[6] PIPER, C. V., and MORSE, W. J. THE SOYBEAN. 329 p., illus. New York. 1923.

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